THE EFFECT OF CERTAIN CARDIOVASCULAR DRUGS ON TISSUE RESPIRATION

DEANE NESBITT CALVERT

ABSTRACT

An investigation was undertaken to examine the effects of injections of ouabain, digitoxin and quinidine upon the tissue respiration of heart and diaphragm of the rat and guinea pig and of brain of the guinea pig.

QO_2 values were determined using samples from the same organ in Krebs Ringer Phosphate (KRP), in Krebs Medium III (KMIII) and suspended in oxygen (HM flasks).

Ouabain caused: a decrease in rat heart respiration in HM flasks, in rat diaphragm respiration in HM flasks, in guinea pig diaphragm respiration in both fluid media and in HM flasks; an increase in guinea pig heart respiration in HM flasks and in guinea pig brain respiration in HM flasks.

Digitoxin caused: an increase in rat diaphragm respiration in KRP and no effect on any other tissue in any medium.

Quinidine caused: a decrease in rat heart respiration in KRP, in rat diaphragm respiration in both fluid media and in HM flasks and a decrease in guinea pig diaphragm respiration in the three media; an increase in guinea pig heart respiration in KMIII but no further effect on the oxygen consumption of the heart of either animal.

Since many of the effects noted were apparent to a more marked degree in the HM flasks than in the fluid media it is suggested that this technique offers a more sensitive method for pharmacological investigation at the cellular level.

THE UNIVERSITY OF ALBERTA

THE EFFECT OF CERTAIN CARDIOVASCULAR DRUGS ON TISSUE RESPIRATION

A DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

FACULTY OF PHARMACY

by

Deane Nesbitt Calvert B.A., B.Sc.

EDMONTON, ALBERTA,

August, 1956

TABLE OF CONTENTS

ACKNOWLEDGEMENT

The author gratefully acknowledges the valuable assistance, the encouragement and the constructive criticism offered by Dean M.J. Huston throughout the course of this investigation.

He wishes to thank the Canadian Foundation for the Advancement of Pharmacy; Smith, Kline and French Interamerican Corporation and the University of Alberta Medical Research fund for financial assistance which made this research possible.

His thanks are also due to Mr. K.E. Moore for technical assistance and to Mrs. K.E. Moore who typed the manuscript.

A note of thanks is extended to Mr. J. Gula for the care of the experimental animals.

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

Investigation into the action of the cardiac glycosides has been proceeding for many decades and gradually our knowledge of their action in cardiac function is accumulating. Chief attention has been directed toward clinical evaluation in heart dyscrasias and although the physiological action of the drugs has been defined to some extent, the basic mechanism of action at the cellular level is by no means understood. With this in mind and with a new tool for pharmacological investigation in our hands, it was decided to attempt to shed further light upon the subject.

Cardiac glycosides have been used by man since the time of the first medical writings and possibly even earlier. The earliest use was probably as poisons and even today some primitive tribes use them for arrow poisons. Squill was used by ancient Egyptians and Romans for a variety of human ills and as rat poison. Although the cardiac glycosides were used empirically for many centuries it was not until 1785 when William Withering published his classic monograph that a beginning was made toward an understanding of the action and utility of digitalis. Withering was not aware that the action of the drug in dropsy was due to its cardiac activity although he knew that the heart was affected. The indiscriminate use of the drug after Withering's publication led it to fall into disrepute until the twentieth century when it was reintroduced into therapy and extensive investigation established it as a specific in the treatment of congestive heart failure.

A few years before Withering wrote on Foxglove a French physician Jean-Baptiste de Sénac from Paris employed cinchona for, as he called it, "rebellious palpitation",(1). This use of the drug seems to have escaped

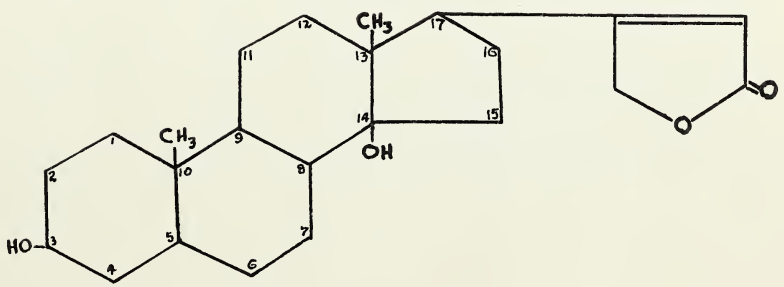
medical attention and it was not until the twentieth century that medical history again records its use for this purpose. Because of the close relationship between quinidine and quinine, its history is linked with that of the latter. Indeed it was the use of quinine as an antimalarial in patients suffering from atrial fibrillation that demonstrated a pharmacological effect on the latter condition. Later investigation showed that quinine was less effective than its dextrorotatory isomer quinidine. Thus quinidine was introduced into twentieth century medicine where it continues to maintain a useful position in the treatment of heart beat irregularities.

II. CHEMISTRY OF THE DRUGS USED IN THIS INVESTIGATION

The glycosides which show cardiac activity are derived from a number of botanical sources and are also found in a venom produced in the skin of certain species of toads. Some of the more important plants which contain the glycosides are Digitalis purpurea, Digitalis lanata, Strophanthus kombé and Strophanthus gratus. The various cardiac principles isolated from these plants are very similar chemically. They consist of a combination of an aglycone or genin with one to four molecules of sugar. The activity of the glycosides rests in the aglycone although the sugar molecules enhance solubility and are thought to increase cell permeability,(2). The sugar molecules may be common monosaccharides, such as glucose, or specific ones, such as digitoxose, found nowhere else except in combination with such aglycones. The genin has a cyclopentanoperhydrophenanthrene nucleus to which is attached an unsaturated 5 or 6 member lactone ring. The lactone ring is essential for the glycoside to have cardiac activity.

1. Digitoxin

Digitoxin has been and continues to be the most widely used of the cardiac glycosides. It yields on acid hydrolysis digitoxigenin and 3 molecules of digitoxose,(3). According to Stoll (4) the structural formula of the genin is as below.



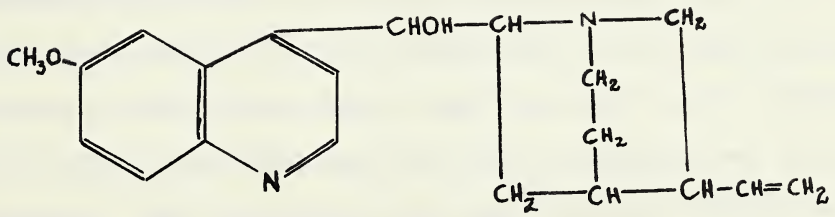
The sugar molecules are attached to the -OH on carbon-3. This glycoside is present in both Digitalis purpurea and Digitalis lanata.

2. Ouabain

Ouabain is the principle glycoside found in Strophanthus gratus and was so named because it was first isolated from the Ouabaio tree,(5). Ouabain departs from the general rule of these cardiac glycosides in being water soluble. This property has made it very valuable for intravenous administration where rapid digitalization is required. The chemical structure of ouabagenin is very similar to that of digitoxigenin with the exception of a $-CH_2OH$ group in place of the $-CH_3$ at carbon 10 and two additional -OH groups at carbon 1 and carbon 5.

3. Quinidine

Quinidine is one of the natural alkaloids of cinchona bark, being the dextrorotatory isomer of quinine.



III. GENERAL PHARMACOLOGY OF THE DRUGS USED IN INVESTIGATION

A knowledge of the general pharmacologic activities of the cardiovascular drugs is essential to an understanding of the mechanism of action of the drugs on the biochemical phenomena being investigated and to an interpretation of the observed effects.

1. Digitalis (Digitoxin and Ouabain)

The main pharmacodynamic property of digitalis is its unique ability to increase the force of myocardial contraction and it is this property which allows it to have beneficial effect in congestive heart failure (1). This increased force of myocardial contraction is brought about by a direct action upon the heart muscle and is independent of other cardiac or extracardiac factors (6). The increase in force of the systolic contraction results in a more complete emptying of the ventricle and improvement in diastolic filling thus improving circulation.

Digitalis has been shown to slow cardiac rate in animals (7). The mechanism is believed to be due to a reflex through the carotid sinus and aortic arch receptors and not through effect on the medullary centres. In studies of atrial fibrillation it has been shown that the conducting tissue of the atrial-ventricular node (A-V node) is rendered less sensitive by digitalis. That the slowing of the heart in atrial fibrillation is due to both vagal and extravagal (direct cardiac action) factors has been shown and the ratio of vagal to extravagal factors depends upon the dose of digitalis. When the full dose of digitalis is administered the extravagal factor appears to be more prominant (8).

Therapeutic doses of digitalis cause no significant change in systemic blood pressure in normal human beings. In animals, toxic doses raise the blood pressure due to direct action on the smooth musculature of the blood vessels and in part to central vasomotor stimulation. Animals receiving the equivalent of therapeutic human doses show no such rise in pressure. In man, with congestive heart failure, digitalis produces no constant effect on the blood pressure. In some cases the pressure is raised, in others lowered, but the majority show no change in arterial pressure.

In congestive heart failure the heart is usually enlarged and the tonic properties of digitalis reduce the cardiac size, which in turn improves the cardiac output. Unless the congestive failure is accompanied by an increase in cardiac size, no increase in cardiac output is noted. There is a two-fold mechanism here in that the strength of the contraction is increased accompanied by a reduction in size.

A diuretic action is noted after digitalis which is secondary to the improvement in circulation and is not due to a direct action of the drug on the kidney. This is demonstrated by the observation that normal persons or animals receiving the drug do not show diuresis. Also persons who have oedema from causes other than congestive heart failure exhibit no diuresis on receiving digitalis. One glycoside, scillaren from squill, is believed to have a direct action on the renal epithelium,(4).

Circulatory changes mediated by the cardiac glycosides have an effect on brain cells but at least part of the activity on the central nervous system is due to direct action. This may be seen by the convulsions produced in animals after administration of large doses of the glycosides.

2. Quinidine

In high concentrations quinidine is a protoplasmic poison but when employed in carefully controlled doses it has a very beneficial effect in a variety of disorders of cardiac rhythm. The changes in rhythm are primarily due to the drug's vagolytic effect on the heart. This effect is similar to, but less pronounced than that of atropine. Because vagal impulses decrease the refractory period and increase the speed of conduction in the atrium, quinidine indirectly produces the opposite effects by its anticholinergic action. Moreover it has a direct action on the atrial muscle which increases the effective refractory period and slows conduction.

In both normal animals and man significant increases in the pulse rate have been found after doses of quinidine,(9,10). Large oral doses have been found to cause a fall of arterial blood pressure in both normal and hypertensive individuals. This fall in blood pressure is believed caused mainly by peripheral vasodilatation,(11).

The basis for the ability of quinidine to prevent or abolish premature systole of atrium or ventricle is its property of depressing myocardial excitability. Simultaneously, the drug decreases myocardial contractility as can be seen after large doses of quinidine when both stroke volume and work per beat are reduced.

Conduction time in the atrium, bundle of His and ventricle is prolonged after quinidine has been administered. Since vagal impulses depress conduction in the A-V node, the anticholinergic action of quinidine opposes somewhat its direct depressive action on this node.

IV. LITERATURE SURVEY

1. The Effect of Certain Ions on the Activity of Cardiac Glycosides

Certain ions, notably calcium and potassium, have been found to affect the action of digitalis on the heart although the picture is still rather confusing. The role of calcium ion on the action of digitalis is, in the opinion of some workers (12,13,14,15), synergistic and indeed Salter, Sciarini and Rubin (16) suggest an assay for cardiac glycosides based on this synergism. Others (17,18) report only additive effects. On the other hand, Nahum and Hoff (19) and Smith, Winkler and Hoff (20) claim neither synergism nor even additive effect. Their work has been criticized however since they administered the calcium to rabbits eighteen hours after the digitalis, and in rabbits, digitalis is claimed to have been excreted before this time (14). That calcium is not needed for digitalis to have cardiac activity has been shown by several workers (21,22). Goodman and Gilman (1) caution against giving calcium intravenously to patients under digitalis therapy but feel the evidence for its relationship with digitalis is incomplete.

Potassium ion plays an important role in heart function and hence any interrelationship between potassium and digitalis is significant. Calhoun and Harrison (23) reported a decrease in potassium content of dog heart when the animals received toxic doses of digitalis but no decrease at lower doses. This finding was supported by Wood and Moe (24) and by Wedd (25) although they noted a slight decrease in potassium content of the heart at therapeutic levels of digitalis. Wood and Moe (24,26) also reported an increase in potassium in the blood and serum in heart lung preparations under digitalis administration. Goodman and Gilman (1) state that increased serum potassium

may counteract, and decreased (below normal) serum potassium accentuate, the effect of the glycosides.

2. The Effect of Cardiac Glycosides on Tissue Metabolism

In order to clarify the review of the literature which deals with the metabolic effects of the cardiac glycosides, the subject has been divided into the following sections: (i) the effect on oxygen consumption; (ii) the effect on metabolism of various metabolites; (iii) the effects on phosphorus metabolism.

(i) The effect on tissue oxygen consumption

One of the earliest reports that cardiac glycosides had an effect on oxygen consumption was that of Victor (27). Using frog ventricle homogenate and Thunberg technique he showed that the depression of oxygen consumption produced by isotonic sucrose could be overcome by ouabain. In Ringer's solution the increase in oxygen consumption was even greater. Macht (28), on the other hand, demonstrated a marked slowing of decoloration after addition of digitoxin to heart homogenate in Thunberg tubes. Gremels (29) using heart lung preparations found that digitalis glycosides lower oxygen consumption of both normal heart lung preparations and in those which have been denervated (in which oxygen consumption is always excessively high). Strophanthin and digitoxin were found by Salomon and Riesier (30) to have no effect on the respiration of isolated frog heart or on respiration of macerated frog, mouse or rabbit heart muscle.

The most significant work in this field has, of course, been done using Warburg technique. Some early investigators, Yavorsky and Reif (31), using guinea pig heart mince in Locke solution with a gas medium of air

found an increase in oxygen consumption when 1% Infusion of Digitalis was added from the side arm of the Warburg vessel. Wollenberger (32) using guinea pig heart slices in Krebs-Ringer Phosphate fortified with glucose showed an increase in oxygen consumption following the addition of ouabain from the side arm, sixty minutes after thermal equilibrium. This stimulation he found to be followed by a depression to below normal levels when the concentration of the drug was high. At lower concentrations of drug a stimulation only in oxygen consumption was noted. Two conditions which he found were necessary for this stimulation of oxygen consumption to occur were the presence of glucose and intactness of the heart cells. A similar diphasic effect on the respiration of heart slices under the influence of Lanatoside C was found by Dunn, Holland and Greig (33). Lévy (34) and Lévy, Schwob and Libert (35) obtained similar results using rat heart slices. However a large dose of the glycoside decreased oxygen consumption without initial stimulation when the drug was tipped in from the side arm. Finkelstein and Bodanski (36) demonstrated an increase in respiration of cat heart slices when scilliroside, ouabain or digitoxin was added to the reaction mixture two hours after it was placed on the apparatus. Glucose was found to be necessary for maximum response although some increase in oxygen consumption was noted when no glucose was present. They found calcium ion essential for the action of the glycosides as have other workers (37,38,39). They did not find the diphasic action noted in Wollenberger's work (32).

Herrmann(38) added ouabain one hour after thermal equilibrium to guinea pig, mouse and rat heart slices and found a diphasic action (stimulation followed by depression) in the case of the first two species but no such effect on rats. This effect was noted whether or not glucose was present. He

administered ouabain to guinea pigs by injection and found a slight increase in respiration. Rothlin and Schoelly (39), using rat heart slices, like Wollenberger (32) found an increase only or a diphasic action on the oxygen consumption depending on the dosage of digitalis or ouabain tipped into the reaction mixture. Unlike Herrmann (38) they were able to show this effect on rat heart slices both when the glycoside was tipped in and when it was administered to the living animal. Equivalent of human therapeutic doses produced a decrease in the oxygen consumption below normal. Nowy and Helmreich (40) also found this depression in oxygen consumption after toxic doses given by injection to rats. Lévy et al (35) showed an increase in oxygen consumption when therapeutic doses were administered to rats. Working with bufagin at high concentrations Doull, Herrmann, Geiling and Dubois (41) were able to show diphasic activity on the respiration of guinea pig heart slices. Using rat heart slices they demonstrated a depression of respiration with high concentrations of bufagin but no effect at low concentrations. Fischer, Huber and Langemann (42) found that convallatoxin, ouabain, strophanthoside and digilanid all slightly increased the oxygen consumption of guinea pig heart slices and slightly decreased the respiration of diaphragm tissue. Using cat and guinea pig heart slices, Langemann, Brody and Bain (43) employing techniques similar to that of Finkelstein and Bodanski (36) found stimulation of respiration when ouabain was tipped in from the side arm two hours after thermal equilibrium, and a stimulation after the slices had been previously depressed by barbiturate. In neither case was the elevation of respiration beyond the initial rates.

Lee (44), using a modified Warburg flask whereby he could measure contraction as well as oxygen consumption, found, with cat papillary muscle,

that ouabain added from the aide arm caused an immediate increase in the height of contractions. However an increase in oxygen consumption did not occur for an hour, reaching a maximum three hours after the addition of the drug. At higher concentrations of the drug similar time ratios were obtained but the intervals were shortened. Hisada and Nakashima (45) using guinea pig heart slices found an increase in respiration in the presence of k-strophanthin with or without the presence of glucose. Washing the slices before adding them to the Warburg flasks weakened the activity of the glycosides. Herrmann, Flamboe and Chen (46) showed that several different cardiac steroids caused a sustained increase in the respiration of cat ventricle slices. The potency of the drugs varied but the overall effect was the same. Hunziker (47) showed that an increase was produced by g-strophanthin, convallatoxin and k-strophanthin in the respiration of guinea pig ventricle slices.

Burdette (48) using human biopsy material demonstrated a well defined increase in respiration following Lanatoside C tipped into the flask which effect was not dependent on the presence of glucose or pyruvate. The same author (49) using C^{14} labelled digitoxin showed an increase in oxygen consumption of human cardiac slices but no conversion of digitoxin to $C^{14}O_2$. Smith, Glassman, Lind, Post, Sohn, and Warren (50) found that ouabain increased the respiration of five day chick embryo hearts in Ringer or Locke solution.

Table I presents a summary of the preceeding literature in an attempt to clarify the rather confusing mass of experimental findings of the effects of cardiac glycosides on tissue oxygen consumption.

TABLE I

SUMMARY OF LITERATURE SURVEY ON THE EFFECTS OF

CARDIAC GLYCOSIDES ON HEART MUSCLE OXYGEN CONSUMPTION

Reference	Animal	Drug	Type of Heart Preparation	Method of Addition of Drug	Medium Used and/or Substrate Present	Effect on Oxygen Consumption	Notes
27	Frog	ouabain	homogenate	-	isotonic sucrose Ringer solution no substrate	depression caused by sucrose overcome increase	Thunberg technique
30	Frog	digitoxin	isolated	-		no effect	
	Frog, Mouse, Rabbit	strophanthin	macerated	-		no effect	
28	Cat, Dog Rabbit Calf, Ox	digitoxin	homogenate	-	no substrate	decrease	Thunberg
31	Guinea Pig	1% infusion of digitalis	mince	tipped in from sidearm	Locke solution (glucose)	increase	gas medium-air
33	Guinea Pig	Lanatoside C	slice			diphasic (increase followed by decrease)	

TABLE I (continued)

Reference	Animal	Drug	Type of Heart Preparation	Method of Addition of Drug	Medium Used and/or Substrate Present	Effect on Oxygen Consumption	Notes
41	Guinea Pig	bufagin	slice	present from onset saturation of medium	Krebs Ringer Phosphate (KRP) with glucose	diphasic	calcium ion needed, lower concentration of bufagin led to increase only in respiration
45	Guinea Pig	k-stroph-anthin	slice	tipped in	no substrate	increase	
47	Guinea Pig	g-strophanthin k-strophanthin convallatoxin	slice	tipped in	no substrate	diphasic	
32	Guinea Pig	ouabain in high concentration	slice	tipped in after 60 minutes	KRP with glucose	diphasic	intactness of cells and presence of glucose found essential to produce these effects
		ouabain in low concentration	slice	tipped in after 60 minutes	KRP with glucose	increase	
38	Guinea Pig	ouabain	slice	tipped in after 1 hour	glucose	diphasic	Also noted when no substrate present
			slice	administered to animal by injection	glucose	slightly increased initially	

TABLE I (continued)

Reference	Animal	Drug	Type of Heart Preparation	Method of Addition of Drug	Medium Used and/or Substrate Present	Effect on Oxygen Consumption	Notes
42	Guinea Pig	ouabain strophanthoside convallatoxin digilanid	slice			increase slightly	
43	Guinea Pig	ouabain	slice	tipped in 2 hours after thermal equilibrium	electrolyte solution with glucose	increase 30-40 minutes after addition	similar results after preliminary barbiturate depression. At no time was stimulation greater than initial rate
40	Rat	strophanthin	slice	Toxic doses administered by injection		decrease	
41	Rat	bufagin	slice	saturation of buffer	glucose	decrease	
				smaller amounts	glucose	no effect	
35	Rat	ouabain	slice	tipped in	none	diphasic	medium low in calcium
		ouabain	slice	injection of non-toxic doses	none	increase	higher dose led to decrease from start

TABLE I (continued)

Reference	Animal	Drug	Type of Heart Preparation	Method of Addition of Drug	Medium Used and/or Substrate Present	Effect on Oxygen Consumption	Notes
38	Rat	ouabain	slice	tipped in one hour after equilibrium	glucose	no effect	
					no glucose	no effect	
39	Rat	digitalin ouabain	slice	tipped in		diphasic-high doses decrease lower doses	
	Rat	digitalin ouabain	slice	administered by injection		therapeutic doses-increase toxic doses decrease	
36	Cat	scilliroside ouabain	slice	tipped in after 2 hours	KRP with glucose	increase	Animal anaesthetized with ether before killing
44	Cat	ouabain	papillary muscle as a whole piece	tipped in	Locke solution (glucose)	increase but only after firm contracture had developed	Special device for measuring contraction as well as oxygen consumption

TABLE I (continued)

Reference	Animal	Drug	Type of Heart Preparation	Method of Addition of Drug	Medium Used and/or Substrate Present	Effect on Oxygen Consumption	Notes
43	Cat	ouabain	slice	tipped in after 2 hours tipped in after barbiturate	electrolyte solution with glucose	increase no greater than initial rates	
46	Cat	several cardiac steroids including ouabain	slice	tipped in		sustained increase	
48	Man	lanatoside C	slices of auricular appendage from biopsy	tipped in	glucose pyruvate	increase both with and without substrate	
50	chick embryo	ouabain	5 day hearts	tipped in	Ringer or Locke solution	increase	
38	Mouse	ouabain	slice	tipped in after 1 hour	glucose	increase	higher dose required to produce this increase than that required for guinea pig. Intermediate between rat and guinea pig.

(ii) The effect on the metabolism of various metabolites

Liebig (51) found that intravenous injection of digitalis principles led to a slight increase in blood sugar and a small decrease in skeletal muscle and heart muscle glycogen in rabbits. Subcutaneous strophanthin did not affect blood sugar and produced only slight decrease in heart muscle glycogen. A similar decrease in glycogen content of liver, skeletal muscle and heart of rats when digitalis was administered was noted by Bomskov, Nikolai, Kaulla and Maurath (52). Kimura and Dubois (53) found with non-toxic doses of digitoxin that there was an increase in formation of liver glycogen in rats; with toxic doses a decrease was shown. He noted no marked differences in distribution of acid soluble phosphorylated intermediates of glycolysis in heart muscle of normal and digitoxin treated animals. Segre (54) found, with ouabain and using homogenates of guinea pig muscle, a decrease in glycogen, glucose-1-phosphate, adenosine triphosphate (ATP) and phosphocreatin and an increase in hexose diphosphate and inorganic phosphate, all of which changes were roughly proportional to the ouabain concentration. Wollenberger (55) using C^{14} labelled glucose, lactate and pyruvate found, in dog myocardium slices under the influence of ouabain, an increase in the rate of production of $C^{14}O_2$ from C^{14} labelled glucose although uptake of the sugar was slowed. Increased $C^{14}O_2$ from lactate, accompanied by an increase in respiration as well as increased uptake of the lactate was also found. Pyruvate on the other hand was not taken up any more quickly but the rate of respiration was about the same as in the presence of glucose or lactate. He suggests that the mechanism of action of the cardiac glycosides is that the system in the heart muscle cell affected by the cardiac glycosides calls upon the lactate as a source of energy. Casasus (56) found a similar reduction in glucose

consumption of rat myocardium with k-strophanthin but an increase in glucose consumption with Lanatoside C. Reiter and Barron (57) using purified enzyme systems could demonstrate no effects on the oxidation of lactate in heart muscle homogenates or slices.

(iii) Effect on phosphorus metabolism

Kimura and Dubois (58) found that digitoxin and ouabain inhibit adenosine triphosphatase (ATPase) activity of normal rat cardiac muscle in vitro and that the amount of inhibition is independent of calcium concentration, while Guerra, Eberstadt and Veerkamp (59) showed that the enzymic liberation of phosphorus in vitro in the system myosin-Na ATP increased if ouabain was added in low concentrations and that the release was increased if the concentration of calcium was increased. Higher concentrations of ouabain produced no increase in the enzymic liberation of phosphorus. Hegglin, Grauer and Munchinger (60) also found that strophanthoside and digilanid increased ATPase activity, while urethane, oxalates, fluorides and copper sulfate, which produce experimentally the so-called energetic heart failure, decrease ATPase activity. In contrast, Helmreich and Simon (61) using k-strophanthoside in similar concentrations to those used by Guerra, showed that inhibition of Ca and Mg activated heart muscle ATPase by the drug is small and not dependent upon the concentration of the glycoside. Langemann (62) moreover showed that k-strophanthoside and purpurea glycoside A had no influence in vitro on the ATPase activity of different cell fractions of heart. Reiter and Barron (57) were unable to show any effect of digitoxin and g-strophanthin on the hydrolysis of ATP with myosin ATPase. Wollenberger (63) was able to show that hearts and heart lung preparations poisoned with ouabain or digoxin were severly depleted of creatin phosphate stores while ATP was slightly lowered. No such change

occured when the dosage of these glycosides was maintained at a low therapeutic level. He suggests then that it is not so much an effect on the myosin ATPase system which the cardiac glycosides produce but a more effective utilization of the ATP energy which is released.

Some investigation has been carried out on the effects of the cardiac glycosides on oxidative phosphorylation. Doull et al (41) found bufagin had no effect on coupled oxidative phosphorylation of heart tissue homogenates. Grisolia (64) showed that concentrations of digitoxin with little effect on respiration or Phosphorus: Oxygen ratios in mitochondrial preparations of guinea pig liver or rabbit heart, markedly potentiate the uncoupling effect of dinitrophenol on oxidative phosphorylation.

Dunn et al (33) found that the diphasic action produced by Lanatoside C on guinea pig heart slice respiration also caused a loss of potassium from the slice. Accompanying this loss of potassium was a stimulation of hydrolysis of acetylcholine. If potassium loss was minimized in some manner then neither the increase in acetylcholine hydrolysis nor the increase in respiration was found. They postulate that the removal of potassium from the heart is the mechanism of the drug's cardiotonic action.

3. The Effect of Cardiac Glycosides on the Brain

Although the study of cardiac glycosides has naturally been directed primarily towards their action on the heart, some attention has been given to their effects on brain.

Wollenberger (32) demonstrated that guinea pig cortex slices were stimulated by ouabain to respire at a higher rate but required a higher concentration than was required to produce an increase in heart respiration. He found brain to be about one-fifth as sensitive as heart. Doull et al (41) found,

upon saturation of calcium-free buffer with bufagin, that a marked inhibition of respiration of homogenates of both rat and guinea pig brain occurred. Concentrations less than saturation of the buffer also produced inhibition. In studies with rat cerebral cortex slices they found an inhibition of respiration produced by bufagin and with guinea pig brain slices, using lower concentrations of bufagin, a similar inhibition was noted. Langemann et al (43) producing a depression of cat and guinea pig brain slices either by a two hour incubation period or by barbiturates, reported no stimulation of respiration after ouabain was tipped in. Likewise no stimulation of respiration was reported when ouabain was added immediately (i.e. without the preliminary depression noted above) to the mixture in the flasks.

Profound changes produced in the glycolytic metabolsim of the brain by the cardiac glycosides are reported by Weese and Wiegand (65). The change assumed the form of a strong inhibition of anaerobicglycolysis. Aerobic lactate formation from glucose is powerfully stimulated according to Wollenberger (66). He explains this on the basis of the preponderance of enzymes for glycolysis in the brain as compared to other tissues. The Kreb's tricarboxylic acid cycle catalysts on the other hand are in relatively lower concentrations in the brain.

4. The Effect of Quinidine on Tissue Metabolism

The effects of quinidine on metabolism have not been extensively investigated.

(i) Effect of quinidine on oxygen consumption

Webb, Saunders and Nakamura (67) using manometric techniques and

with air as the gas phase, tipped quinidine into a reaction mixture containing respiring rat ventricle slices forty minutes after equilibration and found a depression in oxygen consumption occurred. However, when pyruvate, lactate, succinate, or malate had been added previously to the flask, the consequent increase in respiration of ventricle slices was not depressed by quinidine. When glucose was present from the beginning there was no immediate effect upon the oxygen uptake but after one or two hours the respiration was better maintained. Uyeki, Geiling and Dubois (68) found a depression of endogenous respiration of rat cardiac muscle slices when quinidine was tipped into the mixture.

(ii) Effects upon utilization of substrates and upon certain enzyme systems

Webb et al (67) showed a strong inhibition of anaerobic glycolysis in rat heart slices by quinidine and Uyeki et al (68) found a strong inhibition by quinidine of the oxidation of glucose, citrate, fumarate, malate, oxaloacetate, and pyruvate by rat ventricle slices. The latter authors however found no depression of oxidation of α-ketoglutarate and succinate. Using homogenate of rat ventricle, however, the oxidation of pyruvate, α-ketoglutarate and oxaloacetate was depressed in the presence of quinidine. These authors also found that esterification of phosphorus associated with oxidation of succinate, α-ketoglutarate and oxaloacetate was depressed. They suggest that the depressed phosphorylation is a result of inhibition of substrate oxidation and of uncoupling of phosphorylation.

V. STATEMENT OF THE PROBLEM

Most previous investigations undertaken to determine the effect of cardiac glycosides on tissue respiration have been done by adding the drug to the media in the flask in which the excised tissue is placed. It is recognized that this does not represent an *in vivo* situation. The new technique elaborated by Huston and Martin (69) wherein the drug is administered to the living animal and the required tissues subsequently excised and placed in an atmosphere of oxygen superimposed above the fluid media in the Warburg flask would seem to more nearly approach the *in vivo* situation.

It was, therefore, the purpose of this work to examine the effect of a number of cardiovascular drugs on the oxygen uptake of rat and guinea pig heart muscle and diaphragm. The drugs studied were ouabain, digitoxin, and quinidine.

The Huston-Martin method has been shown to be of value in pharmacological investigations by studies using dinitrophenol and sodium aresnite (70). Another facet of the present problem was a comparison of the tissue response in conventional Warburg flasks with the Huston-Martin flasks. Two different media were employed in the Warburg flasks; (1) a buffered electrolyte solution Krebs-Ringer Phosphate and (2) a fortified buffer solution Krebs medium III.

VI EXPERIMENTAL

1. Apparatus and Solutions Used

In order to measure the respiration of tissues in contact with oxygen, it is necessary to have the samples suspended in such a manner as to assure maximal contact of the tissue with the gas. The slices of tissue were spread out evenly on fibre glass mats and placed in wide mouthed flasks designed by Huston and Martin (69)

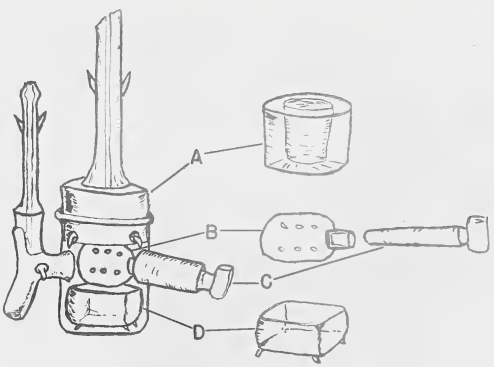


FIGURE I

Figure I is an illustration of the flask. The vessel, of about 19 ml. capacity, is attached to a standard Warburg manometer by a glass adapter (A). A removable tray (D) with four small legs rests on the bottom of the vessel. The mat bearing the tissue is placed on a rotatable paddle (B,C,) above the tray.

Since it was desirable to compare the respiratory rates of tissues suspended in oxygen with those found by more standard procedures, samples of each organ were investigated also in liquid media. Regular Warburg flasks were used for this purpose. Two liquid media were used; Krebs Ringer Phosphate (71), a simple saline solution and Krebs Medium III (72) a fortified solution. The formulas for these solutions are as follows:-

(i) Krebs Ringer Phosphate (KRP)

0.9% (0.154M) NaCl 100

1.5% (0.154M) KCl..... 4

1.22% (0.11M) $CaCl_2$..... 3

2.11% (0.154M) KH_2PO_4..... 1

3.82% (0.154M) $MgSO_4 \cdot 7H_2O$..... 1

0.1M Phosphate Buffer pH 7.4... 12

(17.8g. $Na_2PO_4 \cdot H_2O$; 20 ml. 1N HCl) diluted to 1 Litre

(ii) Krebs Medium III (KMIII)

0.9% (0.154M) NaCl..... 95

1.15% (0.154M) KCl..... 4

1.22% (0.11M) $CaCl_2$..... 3

2.11% (0.154M) KH_2PO_4..... 1

3.82% (0.154M) $MgSO_4 \cdot 7H_2O$..... 1

1.3% $NaHCO_3$..... 3

Na Phosphate Buffer..... 3 (0.1M Na_2HPO_4 (1.78% $Na_2HPO_4 \cdot 2H_2O$)..100 ; 0.1M NaH_2PO_4 (1.38% $NaH_2PO_4 \cdot H_2O$)... 25)

0.16M Na Pyruvate..... 4

0.1M Na Fumarate..... 7

0.16M Na-L-Glutamate 4

0.3M Glucose..... 5

Since the metabolites present in KMIII cannot be stored in solution longer than a week, they were freshly prepared each week. Concentrated stock solutions of the salts were prepared and kept under refrigeration, being diluted before use and added to the metabolites.

2. Method of Handling Animals and Tissues

Albino rats of the Wistar strain weighing 200 to 400 grams and guinea pigs weighing 500 to 800 grams were used throughout the experiments. Male animals were used in all the experimental work since it has been shown by Wollenberger (73) that response of females to the cardiac glycosides differs from that of males.

The method of administration was as follows: ouabain by intraperitoneal (i.p.) injection in 0.9% NaCl solution, digitoxin by subcutaneous (s.c.) injection in 70% ethyl alcohol solution and quinidine by both intraperitoneal and intramuscular (i.m.) injections in acidified water solution. The animals were killed by a blow on the head or by crushing of the cervical vertebrae upon the appearance of toxic symptoms from the drug. The animal was placed in a cold (2-5°C), moist chamber where the tissues to be investigated were quickly excised. Heart and brain tissues were sliced through a template and diaphragm removed in toto and pieces cut parallel to the fibres with scissors. As the tissues were sliced they were placed upon tared pieces of waxed paper or tared fibre glass mats. These were kept in moist petrie dishes in the refrigerated cabinet until all the sections were ready for weighing. The weighing was done on a Gram-atic balance and the tissues immediately removed

to the Warburg flasks. In both normal and treated animals samples were taken from the heart and from either diaphragm or brain of the same animal. Two samples of each tissues were placed in KRP, two in KMIII and two in the Huston-Martin flasks (HM flasks). The tissues spread out on the fibre glass mats were placed on the paddles in the HM flasks and those on the waxed paper removed to the fluid media. After attachment to the manometers, the flasks were placed on the constant temperature bath at 37.9°C where oxygenation was carried out for two minutes. Fifteen minutes were allowed for thermal equilibration. The operation and weighing required about thirty minutes. Measurement of respiration was by the direct method of Warburg at a shaking rate of 120 cycles per minute.

For CO_2 absorption 0.2 ml. of 5% potassium hydroxide solution was placed in the centre well of the standard flasks and was absorbed into filter paper discs placed on the bottom of the HM flasks. Each flask received 1.5 ml. of solution, this being contained in the removable tray (Fig. I-D) of the HM flasks. Readings were taken every ten minutes for the first hour and every twenty minutes for the second hour.

3. Calculation of Results

Warburg manometers record changes in pressure of gas in the flask in microlitres. The flask constant, coupled with the weight of the tissue is used to convert this figure to a standarized QO_2. The QO_2 expresses, in this investigation, ml. of oxygen consumed per gram of tissue (wet weight) per hour. Since the rate of respiration in artificial medium declines with time, the QO2 value for zero time must be obtained by straight line

extrapolation of the rates during the experimental period. The rate of respiration has been depressed by the cold during the operation procedure and returns to a maximum at the conclusion of thermal equilibration. This rate of respiration is therefore the closest approximation to that in situ. The QO_2 value at sixty minutes after thermal equilibration is not comparable to that of the intact animal but gives an indication of the rate of decline of the respiration of a tissue under artificial conditions. It is for this latter reason that sixty minute QO_2 values were recorded.

Mean QO_2 values of each tissue investigated were determined. Any difference from normal values noted after treatment with a drug was tested for significance by the Student "t" test (74,75). The conventional probability of 0.05 was selected as the point of significance.

VII. RESULTS

1. Rat Heart

(i) Normal

Table II shows the normal values obtained for rat heart in KRP, in KMIII and in HM flasks at zero time and sixty minutes thereafter. The mean QO_2 values at zero time are 1.23 in KRP, 1.83 in KMIII and 2.54 in HM flasks. The progressive increase in rate in the two liquid media and the even higher value obtained in the HM flasks is of interest and substantiates the findings of Huston and Martin (69), although the QO_2 values in KRP and in the HM flasks are lower than they report.

(ii) Ouabain

Ouabain is a convenient member of the cardiac glycosides since it is available in a pure form and is readily soluble in water. In the rat a dose of 45 mg./Kg. of body weight was administered by intraperitoneal injection. This dose approximates the LD_{50} for males as given in "The Rat in Laboratory Investigation" (76). At this dosage the animal survived for approximately one-half hour. The first signs of toxicity were noted ten minutes after the injection and took the form of retching type of movements. These became more and more severe with time. Respiration became laboured and a gradual paralysis of the muscle of the limbs occurred, beginning in the hind legs and gradually encompassing the fore-legs. Convulsions were usually noted shortly before death occurred. It was at the stage when convulsions appeared (about one-half hour after administration of the drug) that the animal was sacrificed. Table III shows the QO_2 values obtained for rat heart

after administration of ouabain at zero time and sixty minutes. It may be noted that a significant decrease in respiration was found in the HM flasks at both zero time and at sixty minutes. Although decreases were noted in the liquid media, these changes were not significant.

(iii) Digitoxin USP

Digitoxin USP is defined as either pure digitoxin or a mixture of cardioactive glycoside obtained from Digitalis purpurea and consisting chiefly of digitoxin with a purity of not less than 95%.

With digitoxin a problem of solubility was encountered. It is insoluble in water but quite soluble in hydroalcoholic mixture. Another problem with digitoxin is the slowness of its absorption. Goodman and Gilman (1) state that (in humans) digitoxin has a maximal action four to twelve hours after intravenous injection when a single, full digitalizing dose is given. The dosage tables in "The Rat in Clinical Investigation" (76) give the lethal dose of digitoxin as 100 mg./Kg. by subcutaneous injection when dissolved in 70% alcohol. We administered the drug in 70% alcohol by subcutaneous injection at a dose of 30 mg./Kg. and eighteen hours before killing the animal. Signs of toxicity noted at this time included a slowing in the respiration, closed eyes with a discharge from them, paralysis of the limbs and convulsions. The heart rate was slower by one-quarter to one-third. Table IV shows the QO_2 values obtained at zero time and sixty minutes for rat heart after administration of digitoxin.

Significant differences from normal were not apparent with the exception of a depression of sixty minute value in HM flasks.

(iv) Quinidine Sulphate

Quinidine sulphate is sparingly soluble in water but is soluble in alcohol and in water which has been slightly acidified. Preliminary experiments with the drug dissolved in alcohol indicated that the amount of alcohol necessary for solution produced toxic effects. The drug was therefore administered intraperitoneally in water slightly acidified with sulfuric acid, at a dose of 30 mg./Kg. The results are recorded in Table V.

A second series of experiments was carried out at a dose level of 300 mg./Kg. by intraperitoneal injection. Toxic symptoms were manifest in slow spasmodic respiration and convulsions within fifteen minutes. Experience showed that the animals would die in about half an hour and were therefore sacrificed at twenty minutes after injection. The results are presented in Table VI.

Quinidine caused a decrease in QO_2 values in KRP using either 30 or 300 mg./Kg. at both zero time and sixty minutes. In KMIII the decrease was apparent only at 60 minutes and in HM flasks only after sixty minutes at the high dose level.

TABLE II

RESPIRATION OF NORMAL RAT HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	0.97	2.23	2.59	0.45	0.50	1.70
	1.22	2.44	3.03	0.51	0.60	1.80
	0.95	1.97	2.30	0.50	0.59	1.43
	0.76	2.10	1.87	0.53	0.57	0.90
	0.73	1.38	2.31	0.46	0.82	1.59
	1.60	1.43	2.36	0.66	0.56	1.50
	1.66	1.36	2.50	0.75	0.44	1.31
	1.81	1.60	2.48	1.10	0.51	1.34
	1.11	2.55	3.09	0.64	0.54	1.54
	1.13	1.87	3.59	0.58	0.52	1.77
	1.24	1.63	2.44	0.58	1.35	1.24
	1.15	1.50	2.13	0.80	0.73	1.24
	1.19	1.61	2.50	0.88	0.98	1.44
	1.40	1.83	2.51	0.78	0.71	1.20
	1.61	1.95	2.65	0.74	1.04	1.55
	1.02	1.66	2.29	0.37	1.18	1.72
	1.44	1.73	2.29	0.56	1.02	1.45
	-	2.10	2.65	-	0.83	1.69
	-	-	2.52	-	1.15	1.65
	-	-	3.06	-	-	1.17
	-	-	2.35	-	-	1.60
	-	-	2.33	-	-	1.84
	-	-	-	-	-	1.70
mean	1.23	1.83	2.54	0.64	0.77	1.49
s.d.m.	0.304	0.342	0.364	0.178	0.267	0.234

TABLE III

EFFECT OF OUABAIN 45 MG/KG I.P. ON RESPIRATION OF RAT HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	0.84	1.75	2.80	0.56	0.63	1.24
	0.58	1.61	2.10	0.34	0.47	1.34
	0.72	1.14	2.50	0.41	0.33	1.16
	0.82	1.20	2.96	0.47	0.20	0.95
	0.98	1.31	2.15	0.41	0.54	1.33
	0.96	1.39	2.44	0.48	0.76	1.48
	0.56	1.36	2.06	0.38	0.91	1.12
	0.57	0.93	1.58	0.41	0.56	0.80
	1.16	1.37	2.46	0.68	0.97	1.14
	0.90	2.18	2.02	0.51	0.79	1.16
	1.91	2.93	2.18	0.71	1.30	1.23
	1.35	1.77	2.20	0.79	0.88	1.28
	1.67	1.57	2.67	0.48	0.82	1.27
	1.53	1.86	2.30	0.47	0.47	1.32
	0.93	2.07	2.47	0.42	1.07	1.51
	0.98	1.64	2.64	0.50	0.63	1.71
	1.42	2.23	2.35	0.75	0.69	1.64
	1.41	1.73	2.30	0.80	0.58	1.48
	1.84	-	2.30	0.70	-	1.36
mean	1.11	1.67	2.34	0.54	0.70	1.29
s.d.m.	0.412	0.461	0.305	0.144	0.262	0.215

TABLE IV

EFFECT OF DIGITOXIN 30 MG/KG S.C. ON RESPIRATION OF RAT HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	1.72	1.62	2.68	0.56	0.36	1.12
	1.35	1.50	2.36	0.99	0.85	0.96
	1.28	1.66	2.56	0.73	0.77	1.29
	1.00	1.99	2.80	0.45	1.08	1.34
	1.11	1.71	2.44	0.35	0.44	1.09
	1.41	1.46	2.56	0.40	0.89	1.19
	1.64	1.74	2.06	0.82	0.64	0.83
	1.21	1.53	2.32	0.62	0.83	0.89
	1.52	1.46	2.66	0.84	0.55	1.02
	2.24	1.57	2.58	0.91	1.01	1.16
	1.12	2.15	2.68	0.94	0.90	1.34
	0.94	1.88	2.66	0.70	0.84	1.23
	1.54	1.82	2.72	0.96	0.91	1.29
	1.26	1.35	2.01	0.87	0.42	0.91
	1.16	1.64	2.86	0.75	0.81	1.25
	1.27	1.80	2.49	0.56	0.76	1.26
	1.42	2.24	2.36	0.59	0.96	1.57
	1.17	1.86	2.93	0.86	0.83	1.32
	-	1.70	2.44	-	0.80	1.18
	-	1.42	-	-	0.88	-
	-	1.98	-	-	1.17	-
	-	1.89	-	-	0.92	-
	-	1.93	-	-	1.01	-
mean	1.35	1.74	2.54	0.72	0.81	1.17
s.d.m.	0.298	0.230	0.237	0.193	0.202	0.181

TABLE V

EFFECT OF QUINIDINE 30 MG/KG I.P. ON RESPIRATION OF RAT HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	0.44	1.67	2.28	0.08	0.24	0.98
	0.42	1.88	2.63	0.19	0.31	1.34
	1.03	1.64	2.85	0.53	0.56	1.75
	0.74	2.10	2.83	0.54	0.50	1.33
	1.29	2.43	2.90	0.75	0.57	1.66
	0.96	2.52	2.65	0.57	0.67	1.69
	0.74	1.82	2.57	0.25	0.37	1.34
	0.82	1.85	2.80	0.25	0.61	1.64
	0.84	2.10	2.56	0.31	0.42	1.01
	0.88	1.54	3.31	0.22	0.60	1.82
	0.93	1.24	2.13	0.47	0.30	0.88
	0.54	1.10	-	0.25	0.43	-
mean	0.80	1.82	2.68	0.37	0.47	1.40
s.d.m.	0.240	0.410	0.302	0.19	0.134	0.319

TABLE VI

EFFECT OF QUINIDINE 300 MG/KG I.P. ON RESPIRATION OF RAT HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	0.78	1.87	2.14	0.51	0.45	1.30
	0.73	1.89	3.21	0.45	0.33	1.41
	0.83	2.54	3.16	0.32	1.09	1.92
	0.69	2.35	3.53	0.31	0.91	1.39
	0.89	1.91	2.21	0.14	0.56	1.35
	0.66	2.09	2.89	0.20	0.53	1.60
	0.83	1.87	2.06	0.45	0.35	0.99
	0.77	1.72	2.33	0.42	0.30	0.70
	0.97	1.53	2.68	0.11	0.56	0.96
	1.15	1.73	2.44	0.20	0.38	0.82
	-	2.38	2.47	-	0.37	0.77
	-	2.47	1.70	-	0.38	0.40
mean	0.83	2.03	2.57	0.31	0.51	1.13
s.d.m.	0.138	0.317	0.517	0.136	0.223	0.414

TABLE VII

SUMMARY OF MEAN QO_2 VALUES OF RAT HEART IN NORMAL AND DRUG TREATED ANIMALS

	KRP		KMIII		HM	
	0'	60'	0'	60'	0'	60'
Normal	1.23	0.64	1.83	0.77	2.54	1.49
Ouabain	1.11	0.54	1.67	0.70	S 2.34	S 1.29
Digitoxin	1.35	0.72	1.74	0.81	2.54	S 1.17
Quinidine 30 mg.	S 0.80	S 0.37	1.82	S 0.47	2.68	1.40
Quinidine 300 mg.	S 0.83	S 0.31	2.03	S 0.51	2.57	S 1.13

Table VII summarizes the effects of the drugs mentioned on rat heart respiration. Figure I represents the summary graphically. The graphs show the values for the QO_2 found at zero time and at sixty minutes. The vertical lines on the bars represent the standard deviations. S in both tables and graphs represents a significant difference from normal.

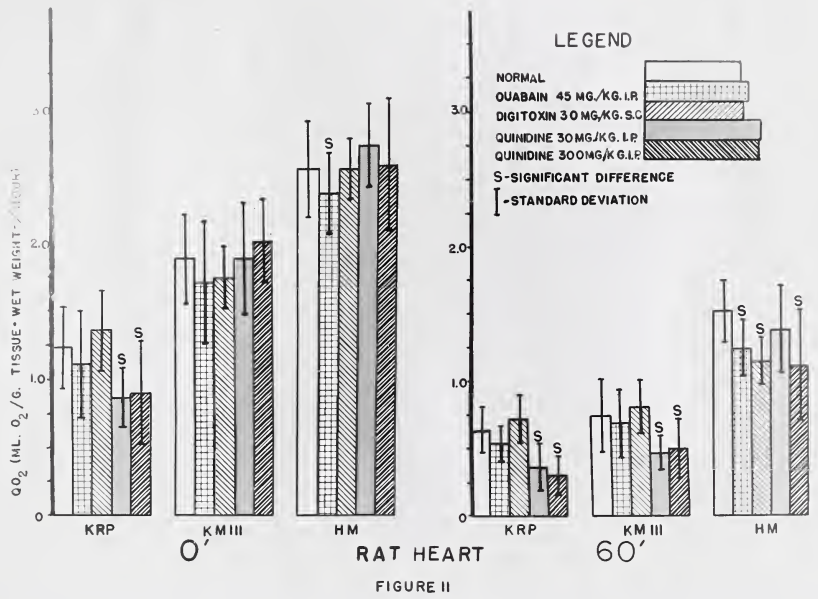


FIGURE II

Figure II The Effect of Ouabain, Digitoxin and Quinidine on Rat Heart Respiration.

2. Rat Diaphragm

(i) Normal

Samples of diaphragm were used from the same animals from which the heart samples were obtained. Table VIII shows the QO_2 values at zero time and at sixty minutes for normal rat diaphragm. In KRP a mean value of 1.11 at zero time was found, in KMIII the value determined at zero time was 1.56 and in HM flasks the same 1.56 value was found. These values correspond quite closely to those found by Huston and Martin (69) with KRP and HM flask values being slightly lower and KMIII slightly higher than their published results.

(ii) Treated animals

The animals from which the heart samples were taken after administration of ouabain 45 mg./Kg. by intraperitoneal injection provided the source of diaphragm tissue for comparison with normal animals. QO_2 values for diaphragm after administration of this drug are presented in Table IX.

Table X shows QO_2 values of rat diaphragm after subcutaneous administration of Digitoxin 30 mg./Kg.

Table XI presents rat diaphragm QO_2 values determined after 30 mg./Kg. of quinidine by intraperitoneal injection and Table XII the values after quinidine 300 mg./Kg. had been administered by intraperitoneal injection.

TABLE VIII

RESPIRATION OF NORMAL RAT DIAPHRAGM

Figures represent Q_{O_2} values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	1.02	1.51	1.84	0.81	0.90	1.24
	0.94	1.15	1.89	0.77	0.69	1.23
	1.02	1.54	1.56	0.64	1.36	1.36
	1.04	1.60	1.62	0.42	1.30	1.51
	1.11	1.38	1.44	0.92	0.78	1.10
	0.84	0.96	1.58	0.49	0.45	1.29
	1.05	1.79	1.66	0.64	1.12	1.53
	1.36	1.60	1.34	0.78	1.10	1.08
	0.99	1.55	1.36	0.65	1.02	1.10
	1.17	1.76	1.59	0.48	0.92	1.39
	1.36	1.55	1.62	0.36	1.19	1.47
	1.18	1.88	1.22	0.78	1.40	1.10
	1.21	1.48	1.25	0.82	1.01	1.14
	0.90	1.30	1.55	0.45	0.85	1.44
	1.04	1.98	1.65	0.60	1.07	1.53
	1.28	2.05	1.31	0.50	0.93	1.14
	1.34	1.50	1.35	0.56	0.98	1.17
	-	1.53	1.71	-	0.99	1.15
	-	-	2.15	-	-	1.51
mean	1.11	1.56	1.56	0.63	1.00	1.29
s.d.m.	0.156	0.264	0.233	0.159	0.228	0.165

TABLE IX

EFFECT OF OUABAIN 45 MG/KG I.P. ON RESPIRATION OF RAT DIAPHRAGM

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	1.12	1.70	1.50	0.89	1.05	1.26
	1.27	1.75	1.52	0.83	1.26	1.33
	1.31	1.82	1.41	0.99	1.09	1.18
	1.48	1.72	1.43	1.02	1.15	1.29
	1.06	1.39	1.18	0.75	1.22	0.90
	1.14	1.71	1.06	0.92	1.40	0.95
	1.04	1.53	1.50	0.88	1.36	0.93
	1.12	1.34	1.60	0.96	1.15	0.97
	1.02	1.46	1.43	0.42	0.94	1.21
	1.04	1.54	1.79	0.64	1.10	1.42
	1.08	1.72	1.56	0.75	1.32	1.36
	-	-	1.62	-	-	1.51
	-	-	1.37	-	-	1.16
	1.18	1.26	1.30	0.82	0.98	1.00
	1.03	0.75	1.02	0.75	0.58	0.99
	0.64	1.09	1.05	0.45	0.90	0.92
	0.77	-	1.05	0.69	-	0.92
	1.69	-	-	0.69	-	-
	1.69	-	-	0.80	-	-
mean	1.16	1.48	1.38	0.78	1.11	1.14
s.d.m.	0.266	0.289	0.224	0.162	0.211	0.195

TABLE X

EFFECT OF DIGITOXIN 30 MG/KG S.C. ON RESPIRATION OF RAT DIAPHRAGM

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	1.12	1.62	1.37	0.91	1.27	1.02
	1.22	1.56	1.57	0.94	1.23	1.13
	1.16	1.21	1.76	0.70	1.11	1.44
	0.98	1.39	1.28	0.59	1.09	1.10
	1.47	1.65	1.52	0.86	1.18	1.14
	1.20	1.68	1.36	0.57	1.12	1.16
	1.10	1.72	1.58	0.75	1.51	1.39
	1.51	1.57	1.43	0.96	1.23	1.31
	1.56	1.75	1.80	0.51	1.21	1.42
	1.79	1.43	1.78	1.05	0.91	1.48
	1.08	1.58	1.69	0.73	1.23	1.52
	1.04	1.52	1.34	0.62	1.11	1.24
	1.23	1.47	1.79	0.78	1.20	1.58
	1.12	1.26	1.54	0.77	1.07	1.33
	1.47	1.86	1.72	1.08	1.16	1.25
	1.58	1.45	1.65	1.17	0.89	1.37
	1.30	1.34	2.02	0.64	0.91	1.58
	1.04	1.10	1.42	0.61	0.73	1.15
	1.49	1.83	1.82	1.15	1.23	1.46
	1.43	1.60	1.62	0.89	1.23	1.38
	1.22	1.77	1.72	0.78	1.47	1.44
	-	1.56	1.80	-	1.33	1.46
	-	2.00	1.47	-	1.41	1.33
	-	1.63	1.41	-	1.04	1.29
mean	1.29	1.56	1.60	0.81	1.16	1.33
s.d.m.	0.221	0.215	0.192	0.199	0.183	0.156

TABLE XI

EFFECT OF QUINIDINE 30 MG/KG I.P. ON RESPIRATION OF RAT DIAPHRAGM

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	1.14	1.36	1.30	0.84	0.94	1.01
	1.14	1.20	1.43	0.74	0.68	1.07
	1.29	1.72	1.24	0.65	0.90	1.19
	1.28	1.70	1.40	0.78	0.93	1.24
	1.73	1.30	1.51	0.65	0.70	1.26
	1.30	1.16	1.26	0.60	0.77	1.09
	1.11	1.70	1.01	0.89	1.10	0.90
	1.08	1.42	1.01	0.69	1.00	0.90
	1.20	1.31	1.19	0.57	0.59	0.92
	1.13	1.67	1.46	0.72	0.87	1.17
	1.79	1.49	2.07	0.84	1.01	1.23
	1.47	1.39	2.05	0.56	1.06	1.40
mean	1.31	1.45	1.41	0.71	0.88	1.12
s.d.m.	0.229	0.193	0.329	0.106	0.155	0.154

TABLE XII

EFFECT OF QUINIDINE 300 MG/KG I.P. ON RESPIRATION OF RAT DIAPHRAGM

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	0.52	0.78	0.52	0.29	0.21	0.21
	0.56	0.84	0.46	0.24	0.28	0.18
	0.51	0.67	0.65	0.27	0.35	0.25
	0.62	0.86	0.79	0.15	0.25	0.41
	0.31	0.76	0.65	0.14	0.24	0.32
	0.24	0.83	0.75	0.14	0.26	0.27
	0.83	1.09	0.64	0.22	0.26	0.23
	0.63	0.90	0.93	0.15	0.13	0.38
	0.97	0.94	0.91	0.27	0.25	0.33
	0.84	1.11	0.75	0.25	0.36	0.27
	0.64	1.01	0.67	0.16	0.38	0.24
	0.57	0.93	0.76	0.18	0.33	0.47
mean	0.60	0.89	0.71	0.21	0.27	0.30
s.d.m.	0.200	0.126	0.132	0.055	0.036	0.083

TABLE XIII

SUMMARY OF MEAN QO_2 VALUES OF RAT DIAPHRAGM IN NORMAL AND DRUG TREATED ANIMALS

	KRP		KMIII		HM	
	0'	60'	0'	60'	0'	60'
Normal	1.11	0.63	1.56	1.00	1.56	1.29
Ouabain	1.16	S 0.78	1.48	1.11	S 1.38	S 1.14
Digitoxin	S 1.29	S 0.81	1.56	S 1.16	1.60	1.33
Quinidine 30 mg.	S 1.31	0.71	1.45	0.88	1.41	S 1.12
Quinidine 300 mg.	S 0.60	S 0.21	S 0.89	S 0.28	S 0.71	S 0.30

Table XIII and Figure III provide a summary of the mean QO_2 values for rat diaphragm. Examination of Figure III and of Table XIII reveal certain significant differences in QO_2 values. After ouabain the respiration in KRP at sixty minutes was significantly increased but significantly decreased in the HM flasks at both zero time and sixty minutes. When digitoxin was administered a significant increase was noted in KRP at both zero time and sixty minutes after equilibration. Similar increase in KMIII at sixty minutes was noted. Quinidine at 30 mg./Kg. produced a significant increase in KRP at zero time and a significant decrease in HM flasks at sixty minutes. At the high dose level, quinidine produced a dramatic decrease at both recorded times in all media.

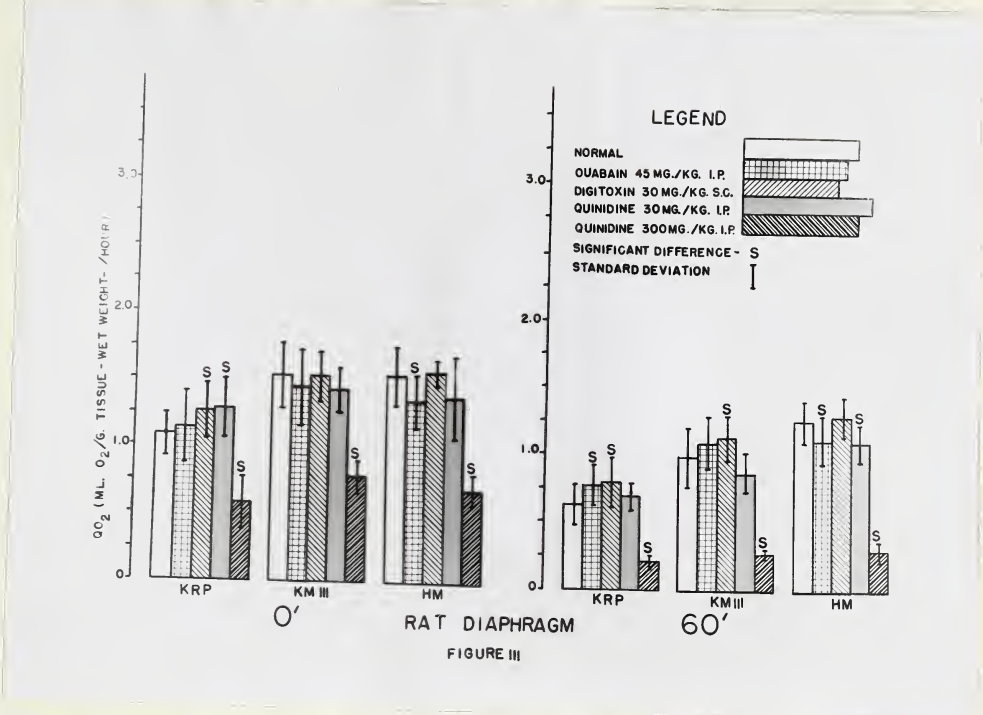


FIGURE III

Figure III The Effect of Ouabain, Digitoxin and Quinidine on Rat Diaphragm Respiration.

3. Guinea Pig Heart

Since several investigations (38,41,77) have shown that the rat is particularily insensitive to the action of the cardiac glycosides and this work seems to bear this out, it was decided to use guinea pigs as well in this investigation.

(i) Normal

Table XIV gives the QO_2 values obtained for normal guinea pig heart slices in the three media at zero time and at sixty minutes. The value at zero time in KRP was 0.50, in KMIII it was 1.62 and in HM flasks was 2.15. These figures would indicate that guinea pig heart respires at a lower rate than does rat heart, the corresponding QO_2 values of which were 1.23, 1.83 and 2.54. It would further indicate that KRP is a particularily poor medium for the study of guinea pig heart respiration.

(ii) Ouabain

Since guinea pigs are much more sensitive to the cardiac glycosides, the dose of ouabain was reduced considerably from that used for the rat (45 mg./Kg.). Doses of 35, 25 and 15 mg./Kg. killed the animals very quickly. At a dose of 10 mg./Kg. the animals survived for 15 minutes and this was the dose used for one series. Symptoms of toxicity noted were similar to those in the rat. Retching movements were pronounced shortly after administration of the drug, followed by progressive paralysis of the limbs from the back forward and later by convulsions.

For the series with brain slices a lower dose of 0.5 mg./Kg. was used. At this dosage the animals survived for 30 to 40 minutes. Tables XV

and XVI present the effects of the two dose levels of ouabain on guinea pig heart slices at zero time and at sixty minutes after equilibration. Statistical examination of these results indicated a significant increase in the QO_2 of the guinea pig heart slices in HM flasks at both zero time and sixty minutes when the dose was 10 mg./Kg. At the lower level (0.5 mg./Kg.) the increase in HM flasks was not significant although a significant increase appeared in KMIII at zero time.

(iii) Digitoxin USP

10 mg./Kg. of digitoxin USP was administered by subcutaneous injection eighteen hours before killing. The digitoxin was dissolved in 70% alcohol. At the time of killing the animal displayed signs of toxicity such as a discharge from the nose and eyes, partial paralysis of the limbs and a reduction in the heart rate by about one-fifth. Table XVII shows the QO_2 values recorded at zero time and at one hour after thermal equilibrium.

No significant changes were produced in the respiratory rates by digitoxin in any of the media at either time interval.

(iv) Quinidine Sulphate

(a) By intraperitoneal injection

The quinidine dissolved in acidified water, was administered by intraperitoneal injection at a dose of 300 mg./Kg. The animals exhibited toxic signs quickly after the injection, marked primarily by convulsions at the slightest stimulus. By the time of killing 15 minutes after injection the animal was very toxic and had become flaccid.

(b) By intramuscular injection

Since the effect of the drug on the diaphragm when administered by intraperitoneal injection might be due in part to a local effect of the solution bathing the diaphragm, a series of experiments were carried out using the intramuscular route. The hearts from the animals so injected were also used. The drug (300 mg./Kg.) was injected into the muscle of the thigh. The injection caused pain and paralysis of the leg. Signs of toxicity did not appear for one-half hour and the animals were sacrificed two hours after injection. Tables XVIII and XIX present the results of the effect of quinidine 300 mg./Kg. by intraperitoneal and intramuscular injection respectively on the respiration of guinea pig heart muscle slices.

At zero time in KMIII there was significant increase when the drug was administered by the intraperitoneal route which was not seen when administration was by intramuscular route. A significant decrease at sixty minutes was noted in the HM flasks but the decrease was not apparent at zero time.

TABLE XIV

RESPIRATION OF NORMAL GUINEA PIG HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	0.52	1.12	2.28	0.13	0.39	0.96
	0.52	1.22	1.96	0.11	0.34	0.70
	0.39	1.55	1.83	0.16	0.25	0.55
	0.43	1.62	2.23	0.24	0.29	0.54
	0.64	1.54	2.60	0.37	0.50	0.94
	0.49	1.60	2.32	0.17	0.35	0.86
	0.55	1.36	2.20	0.20	0.20	0.97
	0.35	1.46	2.14	0.22	0.44	0.72
	0.36	1.59	1.78	0.25	0.28	0.53
	0.49	1.27	2.19	0.16	0.30	0.48
	0.42	1.49	2.28	0.15	0.26	1.03
	0.63	0.94	2.24	0.34	0.15	0.57
	0.59	1.32	2.36	0.30	0.27	0.72
	0.47	1.35	1.67	0.19	0.32	0.79
	0.60	1.91	2.00	0.19	0.30	0.93
	0.58	2.25	1.58	0.29	0.28	0.27
	0.44	2.11	1.79	0.21	0.33	0.45
	0.47	2.04	2.90	0.24	0.31	0.95
	0.54	1.41	2.95	0.37	0.27	0.63
	-	1.57	2.11	-	0.29	0.52
	-	1.79	2.47	-	0.63	0.95
	-	1.92	2.23	-	0.48	1.18
	-	1.74	1.69	-	0.54	1.06
	-	1.69	2.21	-	0.37	1.16
	-	1.90	1.80	-	0.25	1.01
	-	1.87	1.99	-	-	0.74
	-	2.04	2.13	-	-	0.67
	-	-	2.00	-	-	1.02
	-	-	2.50	-	-	1.13
mean	0.50	1.62	2.15	0.23	0.31	0.79
s.d.m.	0.085	0.315	0.331	0.024	0.136	0.243

TABLE XV

EFFECT OF OUABAIN 10 MG/KG I.P. ON RESPIRATION OF GUINEA PIG HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	0.58	1.72	2.36	0.32	0.32	0.82
	0.62	1.60	2.52	0.31	0.49	0.74
	0.40	1.39	2.28	0.22	0.18	0.84
	0.78	1.24	2.26	0.22	0.18	1.17
	0.70	2.02	2.88	0.18	0.40	1.36
	0.56	2.10	2.54	0.20	0.42	1.50
	0.55	1.65	2.87	0.22	0.31	1.09
	0.48	1.78	3.25	0.23	0.35	1.20
	0.52	1.71	2.55	0.19	0.20	0.88
	0.34	1.82	2.24	0.15	0.15	1.13
	0.55	1.86	2.40	0.27	0.15	0.91
	0.59	1.64	2.90	0.33	0.25	1.07
	0.45	1.84	2.75	0.31	0.37	1.16
	0.60	2.04	2.78	0.21	0.31	1.08
	-	1.73	2.26	-	0.49	1.14
	-	1.79	3.09	-	0.43	1.38
	-	1.77	3.10	-	-	1.43
mean	0.55	1.75	2.65	0.24	0.31	1.11
s.d.m.	0.109	0.209	0.319	0.055	0.113	0.217

TABLE XVI

EFFECT OF OUABAIN 0.5 MG/KG I.P. ON RESPIRATION OF GUINEA PIG HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	0.60	2.61	2.73	0.39	0.36	1.10
	0.68	1.21	2.19	0.46	0.40	1.12
	0.66	1.95	2.21	0.21	0.51	1.28
	0.25	2.02	1.84	0.21	2.26	1.30
	0.28	1.84	2.74	0.30	0.39	0.61
	0.54	2.04	1.71	0.25	0.32	0.88
	0.49	1.41	2.60	0.22	0.29	0.35
	0.40	1.83	2.39	0.14	0.53	0.77
	0.36	1.82	2.41	0.16	0.47	1.09
	0.27	1.88	2.13	0.17	0.37	0.77
	0.39	1.45	1.76	0.20	0.63	0.50
	0.58	1.87	2.05	0.23	0.24	0.62
	0.72	1.90	2.66	0.23	0.40	0.79
	-	1.88	2.61	-	0.44	0.91
	-	2.06	-	-	0.33	0.95
	-	-	-	-	-	1.43
mean	0.48	1.85	2.29	0.24	0.40	0.90
s.d.m.	0.159	0.313	0.341	0.088	0.103	0.294

TABLE XVII

EFFECT OF DIGITOXIN 10 MG/KG S.C. ON RESPIRATION OF GUINEA PIG HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	0.25	1.36	1.63	0.11	0.18	0.41
	0.25	1.08	1.83	0.09	0.30	0.98
	0.76	1.54	2.02	0.24	0.47	1.00
	0.31	1.50	2.33	0.21	0.37	0.83
	0.50	1.60	2.42	0.15	0.23	0.97
	0.40	1.32	1.91	0.12	0.32	0.43
	0.50	1.44	2.30	0.09	0.23	0.82
	0.37	1.37	1.87	0.15	0.25	0.61
	0.42	1.91	2.91	0.25	0.19	1.10
	0.42	2.32	3.09	0.19	0.20	0.93
	-	1.34	2.67	-	0.26	1.08
	-	1.14	2.64	-	0.39	1.07
mean	0.41	1.49	2.30	0.16	0.28	0.85
s.d.m.	0.139	0.324	0.442	0.057	0.086	0.233

TABLE XVIII

EFFECT OF QUINIDINE 300 MG/KG I.P. ON RESPIRATION OF GUINEA PIG HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	0.78	2.13	2.07	0.05	0.29	0.79
	0.84	2.19	2.61	0.07	0.35	0.97
	0.52	2.15	1.80	0.24	0.44	0.48
	0.81	1.98	2.22	0.09	0.35	0.59
	0.71	1.75	2.14	0.01	0.32	0.39
	0.53	1.61	2.32	0.28	0.33	0.56
	0.43	1.37	1.93	0.26	0.38	0.20
	0.39	1.59	2.68	0.23	0.37	0.58
	0.44	1.83	1.99	0.24	0.43	0.26
	0.42	1.92	2.37	0.15	0.37	0.54
	0.49	1.38	1.88	0.17	0.38	0.43
	-	1.66	2.29	-	0.39	0.57
mean	0.530	1.80	2.19	0.15	0.37	0.53
s.d.m.	0.224	0.273	0.266	0.098	0.041	0.020

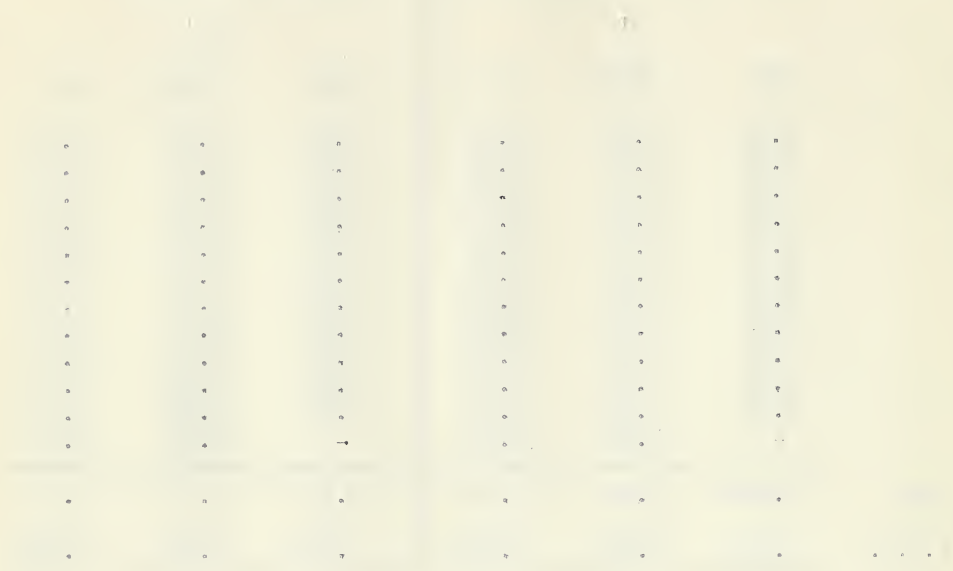


TABLE XIX

EFFECT OF QUINIDINE 300 MG/KG I.M. ON RESPIRATION OF GUINEA PIG HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	0.58	1.41	2.15	0.23	0.30	0.59
	1.04	1.65	2.04	0.40	0.51	0.30
	0.27	1.23	2.08	0.15	0.23	0.31
	0.30	1.66	1.89	0.18	0.28	0.47
	0.43	1.76	1.93	0.25	0.25	0.44
	0.74	1.78	1.84	0.49	0.35	0.30
	0.44	1.67	2.37	0.24	0.26	1.21
	0.48	1.92	2.61	0.26	0.26	1.16
	0.90	1.77	2.64	0.23	0.14	1.00
	0.81	2.02	2.34	0.09	0.42	0.87
	0.50	2.12	2.32	0.35	0.25	0.96
	0.62	1.66	2.05	0.41	0.28	0.74
	-	1.40	2.93	-	0.26	1.10
	-	-	2.50	-	-	1.06
	-	-	2.84	-	-	1.41
	-	-	2.57	-	-	1.47
mean	0.59	1.70	2.32	0.27	0.29	0.84
s.d.m.	0.228	0.239	0.329	0.112	0.089	0.383

TABLE XX

SUMMARY OF MEAN QO_2 VALUES OF GUINEA PIG HEART

IN NORMAL AND DRUG TREATED ANIMALS

	KRP		KMIII		HM	
	0'	60'	0'	60'	0'	60'
Normal	0.50	0.23	1.62	0.31	2.15	0.79
Ouabain 0.5 mg.	0.48	0.24	S 1.85	0.40	2.29	0.90
Ouabain 10 mg.	0.55	0.24	1.75	0.31	S 2.65	S 1.11
Digitoxin	0.41	0.16	1.49	0.28	2.30	0.85
Quinidine 300 mg.i.p.	0.53	0.15	S 1.80	0.37	2.19	S 0.53
Quinidine 300 mg.i.m.	0.59	0.27	1.70	0.29	2.32	0.84

A summary of the effects of the drugs used on guinea pig heart is shown in Table XX and in Figure IV

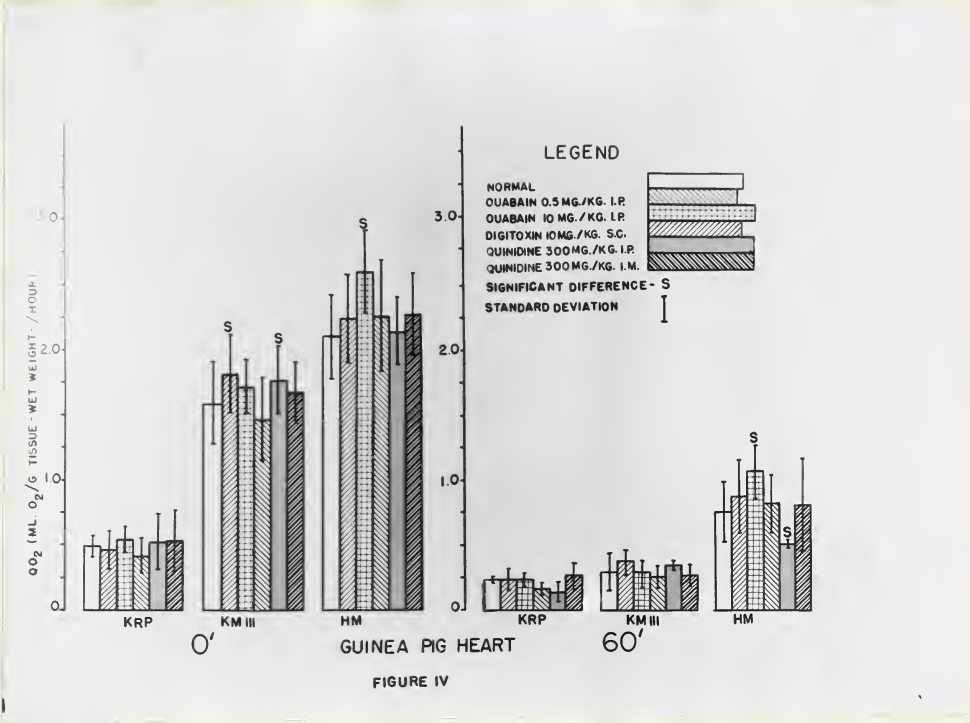


FIGURE IV

Figure IV The Effect of Ouabain, Digitoxin and Quinidine on Guinea Pig Heart Respiration

4. Guinea Pig Diaphragm

(i) Normal

Guinea pig diaphragm is not as thin a muscle as is rat diaphragm and is not as suitable since oxygen may not penetrate as readily to some of the inner cells. Table XXI provides the QO_2 values. In KRP the mean QO_2 for normal guinea pig diaphragm was found to be 0.89, in KMIII to be 1.10 and in HM flasks to be 1.21. In comparison with rat diaphragm corresponding QO_2 values were in KRP 1.11, in KMIII 1.56 and in HM flasks 1.56. The guinea pig values are lower but this is consistent with the findings for heart which were lower for guinea pig than for rat.

(ii) Treated animals

Table XXII presents the QO_2 values obtained for guinea pig diaphragm after intraperitoneal injection of ouabain 10 mg./Kg. Table XXIII provides QO_2 values for guinea pig diaphragm after a subcutaneous injection of digitoxin 10 mg./Kg. Table XXIV lists oxygen consumption values of guinea pig diaphragm after administration of quinidine 300 mg./Kg. intraperitonealy and Table XXV presents QO_2 values for guinea pig diaphragm after quinidine at the same dose level but by intramuscular injection.

TABLE XXI

RESPIRATION OF NORMAL GUINEA PIG DIAPHRAGM

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	0.96	1.05	1.32	0.55	0.70	0.92
	0.76	1.11	1.19	0.39	0.80	0.81
	1.13	1.19	1.52	0.55	0.85	1.03
	1.06	1.26	1.43	0.65	0.99	1.05
	0.84	1.00	1.41	0.50	0.76	1.06
	0.75	1.10	1.49	0.47	0.67	1.09
	0.66	0.94	1.19	0.47	0.53	0.83
	1.14	0.95	1.40	0.43	0.57	1.12
	0.78	0.77	1.02	0.49	0.73	0.73
	0.70	0.69	1.47	0.49	0.63	1.06
	0.48	1.00	1.15	0.35	0.69	0.82
	0.73	1.06	0.98	0.51	0.73	0.89
	0.67	1.03	1.01	0.49	0.74	0.85
	1.05	0.87	0.91	0.73	0.61	0.79
	1.44	1.57	1.25	0.47	0.61	0.97
	0.85	1.65	1.21	0.45	0.83	0.91
	0.63	1.17	1.15	0.29	0.67	0.82
	1.14	1.47	0.99	0.54	0.52	0.73
	1.49	1.39	1.35	0.40	0.65	0.76
	0.84	1.29	0.95	0.65	0.67	0.81
	0.75	0.78	0.96	0.60	0.64	0.81
	0.96	0.84	1.20	0.51	0.61	0.76
	0.74	1.10	-	0.77	0.75	-
	-	1.09	-	-	0.77	-
mean	0.89	1.10	1.21	0.51	0.70	0.89
s.d.m.	0.256	0.241	0.191	0.088	0.103	0.123

TABLE XXII

EFFECT OF OUABAIN 10 MG/KG I.P. ON RESPIRATION OF GUINEA PIG DIAPHRAGM

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	0.84	1.00	0.73	0.46	0.64	0.58
	0.73	1.09	0.84	0.47	0.70	0.67
	0.82	1.12	0.94	0.58	0.87	0.74
	0.87	1.38	1.28	0.63	0.99	0.74
	0.82	0.84	0.82	0.43	0.61	0.60
	0.94	1.00	1.02	0.48	0.68	0.83
	0.53	0.80	1.05	0.34	0.66	0.85
	0.57	0.83	0.99	0.41	0.72	0.88
	0.56	0.72	1.02	0.44	0.58	0.91
	0.63	0.80	1.06	0.49	0.71	0.91
	0.70	1.23	1.24	0.50	0.78	1.03
	0.68	0.85	0.72	0.54	0.67	0.62
	0.70	0.90	0.82	0.28	0.71	0.69
	0.77	-	1.07	0.36	-	0.79
	0.62	0.75	1.32	0.50	0.66	0.98
	0.66	0.80	0.88	0.53	0.69	0.75
	0.80	0.95	0.92	0.40	0.56	0.78
	0.67	1.25	-	0.41	0.60	-
mean	0.72	0.96	0.98	0.46	0.70	0.79
s.d.m.	0.112	0.189	0.173	0.083	0.103	0.127

TABLE XXIII

EFFECT OF DIGITOXIN 10 MG/KG S.C. ON RESPIRATION OF GUINEA PIG DIAPHRAGM

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	0.53	1.25	1.24	0.17	0.64	0.75
	0.77	0.60	1.08	0.65	0.33	0.74
	0.72	1.06	1.04	0.52	0.71	0.70
	1.05	0.78	1.21	0.55	0.56	0.99
	0.92	1.40	1.04	0.47	0.93	0.71
	0.95	1.35	1.03	0.57	1.00	0.66
	0.93	1.17	0.87	0.50	0.55	0.70
	0.94	1.09	1.25	0.63	0.57	0.97
	0.64	1.81	1.36	0.39	0.79	1.11
	1.11	1.85	1.30	0.63	0.75	1.06
	0.85	1.10	0.98	0.40	0.76	0.70
	-	1.14	1.35	-	0.79	0.79
mean	0.86	1.2	1.15	0.50	0.70	0.82
s.d.m.	0.178	0.362	0.159	0.157	0.182	0.161

TABLE XXIV

EFFECT OF QUINIDINE 300 MG/KG I.P. ON RESPIRATION OF GUINEA PIG DIAPHRAGM

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	0.83	0.74	0.45	0.25	0.14	0.21
	0.44	0.66	0.55	0.21	0.34	0.21
	0.34	0.53	0.36	0.19	0.29	0.05
	0.49	0.56	0.20	0.24	0.28	0.15
	0.41	0.88	0.61	0.20	0.37	0.31
	0.47	0.84	0.66	0.35	0.35	0.37
	0.58	0.66	0.39	0.29	0.38	0.22
	0.59	0.54	0.47	0.33	0.34	0.22
	0.47	0.51	0.46	0.24	0.25	0.19
	0.71	0.58	0.93	0.21	0.30	0.59
	0.76	0.60	-	0.27	0.38	-
	-	0.87	-	-	0.55	-
mean	0.55	0.66	0.51	0.25	0.33	0.25
s.d.m.	0.149	0.131	0.224	0.050	0.092	0.139

TABLE XXV

EFFECT OF QUINIDINE 300 MG/KG I.M. ON RESPIRATION OF GUINEA PIG DIAPHRAGM

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	1.13	0.97	0.91	0.64	0.68	0.86
	0.91	1.24	0.95	0.52	0.94	0.76
	0.67	0.75	1.08	0.44	0.55	0.82
	0.54	0.69	0.82	0.40	0.60	0.61
	0.67	0.97	0.98	0.42	0.58	0.84
	0.70	0.84	0.69	0.47	0.67	0.61
	0.92	1.00	0.85	0.58	0.67	0.68
	0.94	0.95	0.90	0.53	0.62	0.73
	0.80	1.02	0.90	0.40	0.59	0.77
	0.75	0.95	0.92	0.46	0.69	0.66
	0.80	1.19	0.80	0.54	0.78	0.83
	0.69	1.06	1.03	0.43	0.76	0.71
	0.87	1.18	0.84	0.26	0.52	0.61
	0.96	1.30	0.69	0.30	0.30	0.59
	0.81	0.89	0.71	0.51	0.66	0.64
	0.74	0.80	0.87	0.50	0.63	0.68
mean	0.81	0.99	0.87	0.46	0.64	0.71
s.d.m.	0.140	0.170	0.110	0.094	0.132	0.089

TABLE XXVI

SUMMARY OF MEAN QO_2 VALUES OF GUINEA PIG DIAPHRAGM IN NORMAL AND DRUG TREATED ANIMALS

	KRP		KMIII		HM	
	0'	60'	0'	60'	0'	60'
Normal	0.89	0.51	1.10	0.70	1.21	0.89
Ouabain 10 mg.	S 0.72	0.46	S 0.96	0.70	S 0.98	0.79
Digitoxin	0.86	0.50	1.22	0.70	1.15	0.82
Quinidine 300mg.ip	S 0.55	S 0.25	S 0.66	S 0.33	S 0.51	S 0.25
Quinidine 300mg.im	0.81	0.46	S 0.99	0.64	S 0.87	S 0.71

Table XXVI and Figure V present a summary of the effects of these drugs on QO_2 values obtained for guinea pig diaphragm. Significantly changed values appeared when the animal was administered ouabain 10 mg./Kg. At zero time the values obtained in both liquid media and in HM flasks were significantly lower than normal. A similar depression was noted with rat diaphragm but the change was significant only in HM flasks. No such depression was noted after the administration of digitoxin to guinea pigs. This lack of change differed from that noted with rat diaphragm where a significant increase was seen at zero time in KRP and at sixty minutes in both KRP and KMIII. Like rat diaphragm a depression was noted in all media at both recorded times when quinidine 300 mg./Kg. was injected intraperitoneally. When the route of administration was changed to intramuscular however, significant changes were apparent only in KMIII at zero time and in HM flasks at both zero time and sixty minutes.

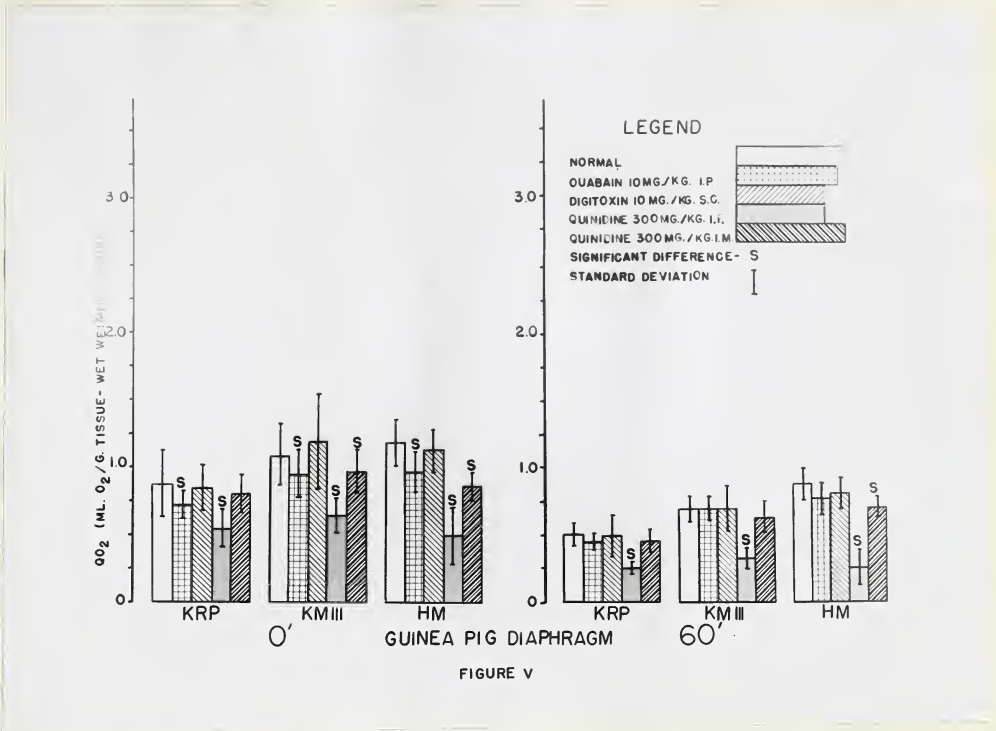


FIGURE V

Figure V The Effect of Ouabain, Digitoxin and Quinidine on Guinea Pig Diaphragm Respiration

5. Guinea Pig Brain

Since the toxic symptoms of ouabain would appear to be due at least in part to a central action, it was thought of interest to investigate the effect on respiration of brain slices using the Huston-Martin technique. Wollenberger (32) has reported an increase in respiration of guinea pig brain when ouabain was added *in vitro* although other workers (41,43) have not. A lower dose of ouabain than that used in the heart and diaphragm experiments was employed to permit a longer time for penetration to, and contact with the brain. A dose of 0.5 mg./Kg. was administered by intraperitoneal injection. Symptoms of toxicity similar to that reported for the higher dose levels were seen although a half hour elapsed before they were fully developed. The animals were killed one half hour after injection. The brain was sliced through a template and slices were as far as possible taken from the cortex. The results obtained with a control series not receiving ouabain are recorded in Table XXVII. In KRP the QO_2 was 1.75, in KMIII 2.30 and in HM flasks 2.85. It will be noted that although the readings at zero time were highest in the HM flasks the rate had fallen off at the end of an hour to a much lower level than in either of the solutions. A similar situation is reported by Huston and Martin (69) when rat brain was used. Here again the values were highest at zero time in HM flasks but had fallen off more quickly than in the fluid media. The QO_2 values for guinea pig brain are somewhat higher than the corresponding values reported by these authors for rat brain.(KRP-1.39, KMIII-2.21, HM flasks-2.77) Table XXVIII presents the results after the administration of ouabain.

TABLE XXVII

RESPIRATION OF NORMAL GUINEA PIG BRAIN SLICES

Figures represent QO_2 values in ml. O2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	1.78	2.19	2.72	1.42	1.88	0.86
	1.75	2.01	2.66	1.22	1.73	0.91
	1.68	2.36	2.58	1.24	1.83	0.91
	1.82	2.15	2.76	1.40	1.62	1.12
	1.74	2.56	3.38	1.41	1.82	0.99
	1.63	2.12	2.94	1.30	2.15	0.78
	1.75	2.22	2.42	1.39	1.83	1.04
	1.47	2.53	3.35	1.16	2.03	0.98
	1.50	1.90	2.35	1.20	1.63	1.04
	1.64	2.51	2.90	1.22	2.15	0.70
	1.67	2.90	2.68	1.34	1.88	0.86
	1.53	2.49	2.78	1.17	1.90	1.40
	2.12	2.11	3.50	1.10	1.64	1.07
	1.91	2.11	-	1.13	1.65	-
	2.14	-	-	1.51	-	-
	1.90	-	-	1.29	-	-
mean	1.75	2.30	2.85	1.28	1.84	0.97
s.d.m.	0.189	0.261	0.348	0.116	0.172	0.168

TABLE XXVIII

EFFECT OF OUABAIN 0.5 MG/KG ON RESPIRATION OF GUINEA PIG BRAIN SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	0'			60'		
	KRP	KMIII	HM	KRP	KMIII	HM
	1.56	2.40	2.96	0.90	2.15	0.96
	1.52	1.95	3.23	0.82	1.62	1.04
	0.97	2.10	3.28	1.35	1.92	1.29
	0.93	2.19	3.26	1.30	2.08	1.21
	2.10	2.21	3.76	1.18	1.89	0.90
	2.01	2.33	3.61	1.36	1.79	1.04
	1.91	2.38	3.61	1.43	1.77	1.22
	1.81	2.53	3.50	1.39	1.71	1.18
	1.63	2.12	2.96	1.37	1.87	0.96
	1.61	1.93	3.25	1.40	1.69	1.02
	1.68	2.07	2.96	1.40	1.81	1.16
	1.56	2.10	3.50	1.22	1.91	1.33
	1.62	1.87	3.07	1.30	1.53	1.04
	1.49	2.11	3.10	1.23	1.82	1.04
	1.58	2.31	2.83	1.41	1.88	0.99
	1.54	2.45	-	1.31	1.88	1.11
mean	1.60	2.19	3.26	1.27	1.83	1.09
s.d.m.	0.300	0.188	0.274	0.172	0.151	0.298

TABLE XXIX

SUMMARY OF MEAN QO_2 VALUES OF GUINEA PIG BRAIN

IN NORMAL AND DRUG TREATED ANIMALS

	KRP		KMIII		HM	
	0'	60'	0'	60'	0'	60'
Normal	1.75	1.28	2.30	1.84	2.85	0.97
Ouabain 0.5 mg.	1.60	1.27	2.19	1.83	S 3.26	1.09

Table XXIX gives a summary comparing normal and experimental results and Figure VI shows the comparison graphically. A statistically valid increase was noted only in HM flasks at zero time.

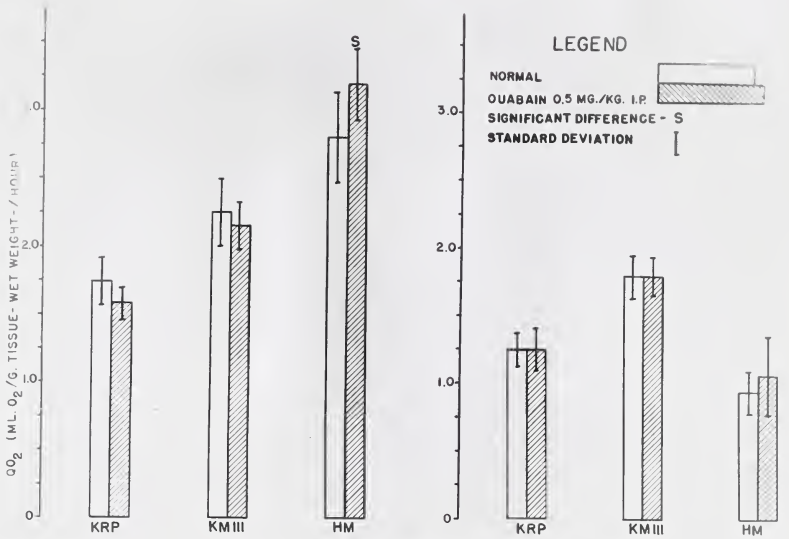


FIGURE VI

Figure VI The Effect of Ouabain on Guinea Pig Brain Respiration.

VIII. DISCUSSION

A discussion of these results must be prefaced by some comments on the procedure proposed by Huston and Martin (69) for the determination of tissue respiration in contact with oxygen. The *in vitro* evaluation of the pharmacological action of drugs at the cellular level after administration of the drug *in vivo* is complicated in standard Warburg methods by such factors as modification of the drugs and tissue metabolites by the liquid suspension medium. Cellular effects demonstrated by the addition of drugs *in vitro* may or may not represent the response in the intact animal, particularily in view of possible differential tissue distribution and sensitivity. Huston and Martin (69) showed that tissue respiration can be measured with the tissues suspended in a gaseous phase of oxygen on fibre glass mats. This technique avoids the variations due to different liquid media and permits quantitative assessment *in vitro* of the tissue effects of the drugs administered to the intact animal.

These workers applied this technique to an examination of the effects of dinitrophenol and of sodium arsenite on the respiration of several rat tissues and showed that there were marked quantitative and even some qualitative differences between the results obtained with tissues on mats and in liquid media. It is their contention that the results in oxygen more nearly represent the *in situ* response than the results with the tissues in artificial liquid or when the drug is added *in vitro*.

Rodnight and McIlwain (80) compared rates of respiration of brain, kidney, diaphragm and liver without added media and in olive oil, light paraffin and silicone fluid. In each case respiration rates were higher

initially than those observed in saline which were run at the same time. They found that unless glucose was added that the rate of respiration fell quite rapidly after 30 minutes. This was particularly true in brain cortex slices.

Some advantages to the technique of administering the drug to the animal and examining the tissues in oxygen would appear to be:-

(a) the drug has been administered in vivo and the distribution and response has been that governed by the intact animal; adding the drug from the side arm in vitro presents a completely artificial situation;

(b) the drug has not been diluted or extracted from the tissues by a liquid medium;

(c) variable influences due to ions or metabolites in the medium are avoided.

Disadvantages or limitations of the procedure may be summarized as follows:-

(a) once the tissue is placed on the mat further drugs and/or metabolites cannot be added to it. The technique therefore does not lend itself to an examination of substrate phenomena;

(b) the tissue cannot act as its own control as is the case when the drug is added from a side arm. It is necessary to run control series of non-treated animals;

(c) a not too serious disadvantage and one which is inherent in all tissue respiration studies is that once the tissue is removed from the body it progressively departs from physiological normalcy. Many factors are involved not all of which are known. Some of the more obvious factors are loss of hormone and nervous control, limitation of supply of metabolites and ions and accumulation of metabolic end products.

However, since the primary interest is the effect of the drug on the tissue at the time it is removed from the body, that is the in vivo effect, this disadvantage is not serious.

(d) a possible disadvantage is that the tissue in contact with oxygen and not supplied with nutriment may burn itself up. This situation would be indicated by a more rapid fall in slope of the graph and at the end of an hour the respiration rate might be expected to be below that of the tissues in fluid. This was noted only in the case of brain slices, a tissue well known to utilize carbohydrate at a very high rate.

These disadvantages are minimized by the procedure of extrapolation to zero time. The rate of respiration has been reduced by the cold during the preliminary manipulations and returns to a maximum at the conclusion of equilibration which is the point of extrapolation. This figure, so obtained would appear to most closely approximate the in vivo situation.

1. The Effect of Ouabain

(i) Rat Heart (Table VII Page 37)

Several workers have found rat heart to be resistant to the action of the cardiac glycosides (38,41,77). From Table VII it can be seen that a slight depression of respiration occured in the two liquid media, although it was not statistically significant. A more marked depression was seen in the HM flasks and this lowering was significant at both zero time ($p<0.05$) and sixty minutes ($p<0.005$). Comparison of these results with those of other

workers is difficult in view of the fundamental difference in technique. Lévy (34,35) found an increase in rat heart slice respiration when the drug at low concentrations was tipped into the reaction mixture, but found a depression at high concentrations. Administration of non-toxic doses (1.5mg./Kg.) by injection caused an increase in the oxygen consumption above normal. Depression similar to that reported by Lévy was found by Doull et al (41) using bufagin at high concentrations and by Rothlin and Schoelly (39) using ouabain and digitalin at high concentrations. These latter workers also report that administration of toxic doses before killing the animal produced a depression of oxygen consumption. Our work, which showed a depression with toxic doses of ouabain, would therefore be in agreement with their findings.

(ii) Guinea Pig Heart (Table XX Page 56)

Ouabain 10 mg./Kg. caused an increase in the oxygen consumption of guinea pig heart slices in the three flasks. This increase was again significant ($p<0.005$) only in the HM flasks. The respiratory rate was still significantly elevated ($p < 0.005$) at sixty minutes while in the fluid media the QO_2 had dropped to the same level as in control animals. Somewhat similar results have been reported by other workers using different techniques: Wollenberger (32) and Dunn (33) found a diphasic action (increase followed by a decrease) with guinea pig heart slices to which high concentrations of ouabain and Lanatoside C had been added from the side arm. Herrmann (38) administering the drug to guinea pigs found a stimulation in respiration of heart slices. Stimulant activity on the cardiac muscle slices was noted

by most workers after they had tipped the glycoside into the reaction mixture from the side arm of the Warburg vessel after a time interval, from thermal equilibration, of up to two hours (32,36,38). At a time such as two hours after removal of the tissue from the body it would appear to be extremely hazardous to apply the findings to an in vivo situation. Added substrate has been stressed as an essential for cardiac glycoside stimulation of respiration of heart slices by certain workers (32,36). Others (38,45) have found stimulation without the presence of substrate. Our results at 10 mg./Kg. dosage would seem to indicate that added substrate is not essential since significant increase occured in the HM flasks and no significant increase was noted in KMIII where substrate is present. However at the lower level (0.5 mg./Kg.) a significant increase in KMIII which was not present in the HM flasks tends to substantiate the need for substrate. It may be that the dose level has an effect on an apparent need for substrate.

The observed decrease in rat heart slice respiration and the increase in guinea pig heart slice oxygen consumption in the HM flasks is not surprising in view of the observations of other workers. Reference to Table I will show that the rat is resistant to the action of the cardiac glycosides (38,41,77), while the guinea pig is susceptible to their effects on heart slice respiration (32,33,38,41). Other species also respond in different ways to these drugs, mouse has been noted to be intermediate in susceptibility (38) while cat (46,43,36) as well as humans (48) respond with a sustained increase in oxygen consumption. Thus it may be assumed that part of the difference in the effect of ouabain noted in this work was due to a species difference. Another factor which must be considered, however

is the dose level used. Since high doses have been observed to cause a depression of rat heart slice respiration (39,40,41), the amount of glycoside which was administered to rats may account for the depression noted in the HM flasks.

(iii) Rat Diaphragm (Table XIII Page 45)

The significant depression ($p < 0,025$) of rat diaphragm respiration by ouabain in the HM flasks and not in the other media would appear to be an indication of the greater sensitivity of this technique. One of the advantages claimed for the Huston-Martin technique (no dilution of the drug by the fluid medium) is probably demonstrated here, for the concentration of ouabain which has reached the tissue before killing is the same since all samples came from the same animal. Dilution by the fluid medium to an ineffective level therefore probably accounts for the lack of effect. The significant increase ($p < 0.01$) at sixty minutes in KRP is probably an anomaly and it must be kept in mind that in interpreting these results, in the light of drug action in the intact animal, chief emphasis must be placed on the values at zero time.

It is interesting to note that ouabain produced a depressant action on the respiration of both heart and diaphragm muscle when the tissue is in contact with oxygen.

(iv) Guinea Pig Diaphragm (Table XXVI Page 64)

In the work using guinea pig diaphragm the respiration is depressed significantly in all three media, in KRP ($p < 0.005$), in KMIII ($p < 0.025$)and

in HM ($p < 0.005$). In comparing this result with that on rat diaphragm it is to be noted that although a much lower dose was used in the guinea pig the depression was more marked (in HM flasks 19% reduction with guinea pig and 12% reduction in rat). Depression of oxygen consumption of guinea pig diaphragm by ouabain was also noted by Fischer et al (42).

A comparison of the effect of ouabain on guinea pig heart and guinea pig diaphragm oxygen consumption shows an increase in heart and a decrease in diaphragm. The explanation probably lies in a difference in sensitivity of the two tissues to ouabain and this work may constitute some evidence for the claimed tissue specificity of the cardiac glycosides.

(v) Guinea Pig Brain (Table XXIX Page 69)

The symptoms noted in the animal when ouabain was administered suggested the possibility of some effects on the central nervous system. The convulsions which occured and the retching movements could be of central origin. Goodman and Gilman (1) mention that the convulsions are due to action on the central nervous system although the retching movements origin is still in doubt. These are also attributed to central action by Sollmann (78).

At a dose level of 0.5 mg./Kg. a significant ($p < 0.005$) increase in respiration was noted in the HM flasks but not in fluid media. This would appear to indicate a greater sensitivity of this technique for pharmacologic examination of drug action on the brain. Wollenberger (32) noted that brain cortex slices of guinea pigs were stimulated by ouabain but only at higher concentrations than were effective for stimulation of

the respiration of heart slices. Doull et al (41) on the other hand found no such stimulation but only a depression with both rat and guinea pig brain. Langemann et al (43) also reported no stimulation of guinea pig brain slice respiration when ouabain was tipped in after preliminary depression in respiration produced by a two hour incubation or by addition of barbiturates to the flask containing the slices.

2. The Effect of Digitoxin

(i) Rat Heart (Table VII Page 37)

The lack of demonstration of any significant effect by digitoxin in this work is possibly not surprising in view of the well known resistance of this animal to cardiac glycosides. Solubility, time factors and dosage may also be involved.

(ii) Guinea Pig Heart (Table XX Page 56)

The complete lack of significant changes in the respiration of guinea pig heart is surprising in view of reported increases by other workers (42). Differences in technique , dosage, and time factors may well account for this disagreement.

(iii) Rat Diaphragm (Table XIII Page 45)

The significant increase in rat diaphragm respiration at both zero ($p < 0.005$) and sixty minutes ($p < 0.005$) KRP after subcutaneous injection of digitoxin is puzzling. In the light of our present knowledge

and data no adequate explanation is available and the disagreement may be an artifact.

(iv) Guinea Pig Diaphragm (Table XXVI Page 64)

Digitoxin produced no significant change in diaphragm in either of the fluid media or in the HM flasks. The lack of response is not surprising since several workers have noted only slight decreases (42) or no effect upon diaphragm or skeletal muscle (79,32).

3. The Effect of Quinidine

(i) Rat Heart (Table VII Page 37)

At both 30 mg./Kg. and 300 mg./Kg. a significant depression of rat heart slice QO_2 values in KRP at zero time and sixty minutes was noted. (30 mg. zero time ($p < 0.005$), sixty minutes ($p < 0.005$), 300 mg. zero time ($p < 0.005$) sixty minutes ($p < 0.005$)). This is in agreement with Webb et al (67) and Uyeki et al (68) who demonstrated a depression in respiration of rat ventricle slices after tipping quinidine from the side arm. This depression was not noted in the presence of substrate which is also borne out in this work by the lack of depression or significant change in KMIII at zero time. Webb et al (67) further contend that in the presence of glucose, respiration is better maintained for one to two hours. This work disagrees somewhat in that in KMIII (in which glucose is present) a significant depression was noted at sixty minutes at both dose levels (30 mg./Kg. ($p < 0.005$) and at 300 mg./Kg. ($p < 0.005$)). On the basis of present information it is

difficult to explain a decrease ($p < 0.005$) at sixty minutes in HM flasks at the higher dose level which is not present at the lower dosage. Perhaps the level of the drug reaching the heart at the higher dosage causes tissue changes which in turn cause a reduction in oxygen consumption only after prolonged contact with the tissue.

(ii) Guinea Pig Heart (Table XX Page 56)

Quinidine at 300 mg./Kg. by intraperitoneal injection had a significant effect ($p < 0.005$) on guinea pig heart only in KMIII at zero time where an increase was noted and in HM flasks at sixty minutes where a decrease was seen ($p < 0.005$). This latter is the same effect as seen in HM flasks at sixty minutes with rat heart. It would appear that the nature and amount of substrate available to the tissue may have an important influence upon the respiratory rate at any specific time. Our data do not supply sufficient information to put forward any hypothesis.

(iii) Rat Diaphragm (Table XIII Page 45)

30 mg./Kg. of quinidine by intraperitoneal injection caused a significant increase ($p < 0.01$) at zero time in KRP. No such change was noted in the other media and no adequate explanation can be offered for this increase.

At 300 mg./Kg. rat diaphragm is significantly depressed in the two liquid media and in HM flasks at both zero time and sixty minutes ($p < 0.005$ in all cases).

(iv) Guinea Pig Diaphragm (Table XXVI Page 64)

A similar depression in all media at both recorded times was noted in guinea pig diaphragm at a dose of 300 mg./Kg. by intraperitoneal injection

($p < 0.005$ in all cases). In order to investigate the possible effect of the route of administration a series was run in guinea pig using intramuscular injections. Once again depression was noted which was significant in KMIII at zero time ($p < 0.05$) and in HM flasks at both zero time ($p < 0.005$) and sixty minutes ($p < 0.005$). The depression noted in this instance, while still significant, was not as marked as that from an intraperitoneal injection. This may mean that part of the effect by intraperitoneal injection was from bathing of the tissue by the injection fluid, however it may be due to faster absorption by this route and a consequent higher level of drug reaching the tissue by normal circulatory means.

4. General Comments

The aim of this research was to test the effect of certain cardiovascular drugs on tissue respiration and to examine the Huston-Martin technique as a tool for pharmacological investigations.

Our results demonstrate that while these drugs do influence tissue respiration no over-all pattern is apparent. Since the level of response at zero time is believed to represent most accurately the situation in the animal, chief emphasis should be placed on this figure in evaluating in vivo effect. A summary of the response in relation to the controls at this time is presented in Table XXX and this relationship is seen graphically in Figures VII and VIII.

It is of interest to compare the effects of the drugs on heart

TABLE XXX

MEAN QO_2 VALUES OF DRUG TREATED ANIMALS EXPRESSED AS A PERCENT OF NORMAL MEAN QO_2 VALUES AT ZERO TIME

	Rat Heart	Rat Diaphragm	Guinea Pig Heart	Guinea Pig Diaphragm	Guinea Pig Brain
KRP Normal	1.23	1.11	0.50	0.89	1.75
Ouabain 45 mg./Kg.	90.2	104.5	-	-	-
Ouabain 10 mg./Kg.	-	-	110.0	S 80.9	-
Ouabain 0.5 mg./Kg.	-	-	96.0	-	91.4
Digitoxin 30 mg./Kg.	109.8	S 116.2	-	-	-
Digitoxin 10 mg./Kg.	-	-	82.0	96.6	-
Quinidine 300 mg./Kg.i.p.	S 67.5	S 54.1	106.0	S 61.8	-
Quinidine 300 mg./Kg.i.m.	-	-	118.0	91.0	-
Quinidine 30 mg./Kg.	S 65.0	S 118.0	-	-	-
KMIII Normal	1.83	1.56	1.62	1.10	2.30
Ouabain 45 mg./Kg.	91.3	94.9	-	-	-
Ouabain 10 mg./Kg.	-	-	108.0	S 87.3	-
Ouabain 0.5 mg./Kg.	-	-	S 114.2	-	95.2
Digitoxin 30 mg./Kg.	95.1	100.0	-	-	-
Digitoxin 10 mg./Kg.	-	-	92.0	110.9	-
Quinidine 300 mg./Kg.i.p.	110.9	S 57.1	S 111.1	S 60.0	-
Quinidine 300 mg./Kg.i.m.	-	-	104.9	S 90.0	-
Quinidine 30 mg./Kg. i.p.	99.5	92.9	-	-	-
HM Normal	2.54	1.56	2.15	1.21	2.85
Ouabain 45 mg./Kg.	S 92.1	S 88.5	-	-	-
Ouabain 10 mg./Kg.	-	-	S 123.3	S 81.0	-
Ouabain 0.5 mg./Kg.	-	-	106.5	-	S 114.4
Digitoxin 30 mg./Kg.	100.0	102.6	-	-	-
Digitoxin 10 mg./Kg.	-	-	107.0	95.0	-
Quinidine 300 mg./Kg.i.p.	101.2	S 45.5	101.9	S 42.1	-
Quinidine 300 mg./Kg.i.m.	-	-	107.9	S 71.9	-
Quinidine 30 mg./Kg. i.p.	107.0	90.4	-	-	-

S indicates a significant difference from normal

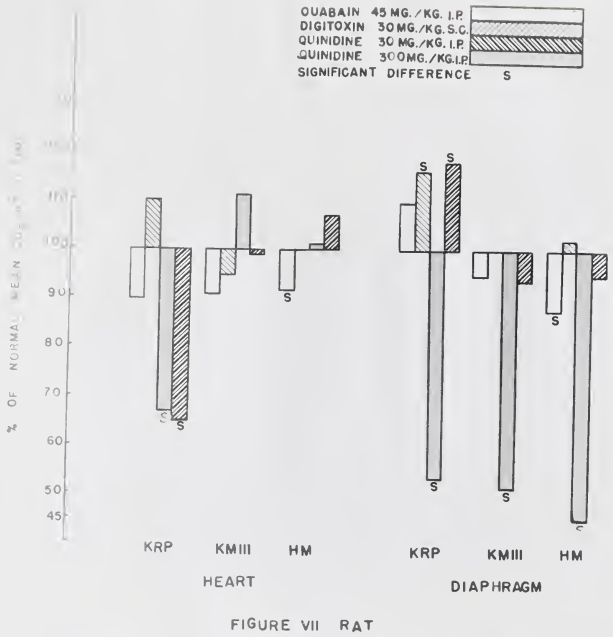


FIGURE VII RAT

Figure VII The Effect of Ouabain, Digitoxin and Quinidine on Rat Heart and Diaphragm Respiration expressed as a percent of mean normal QO_2 values at zero time.

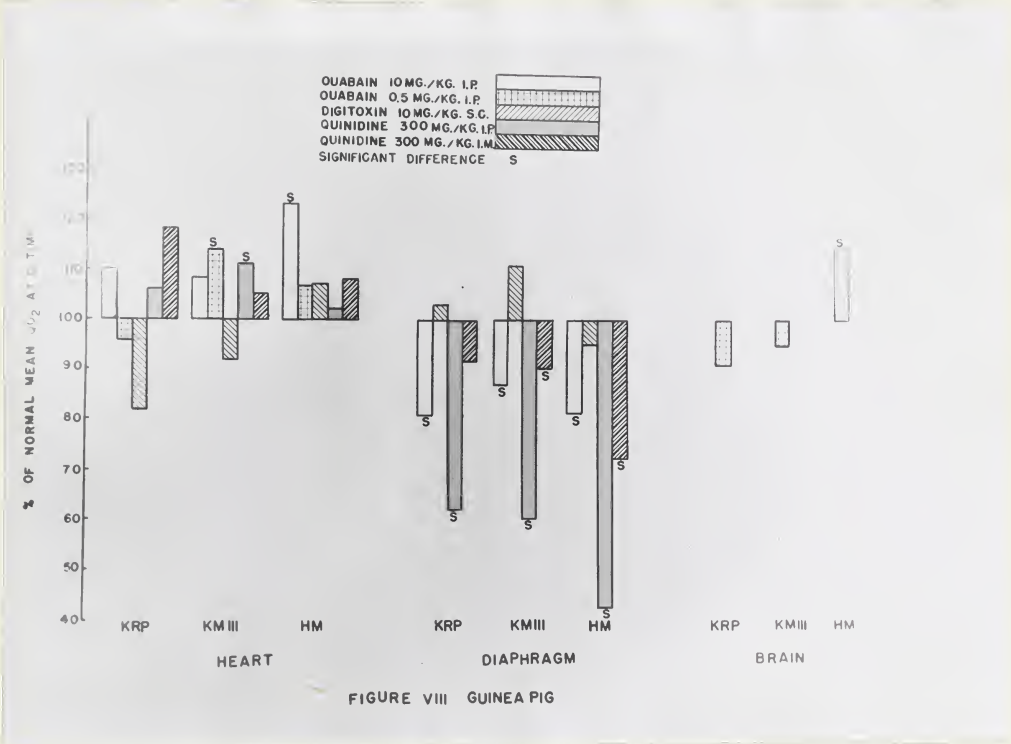


FIGURE VIII GUINEA PIG

Figure VIII The Effect of Ouabain, Digitoxin and Quinidine on Guinea Pig Heart, Diaphragm and Brain Respiration expressed as a percent of mean normal QO_2 values at zero time.

with those on diaphragm. While ouabain caused an increase in guinea pig heart respiration in KMIII and HM flasks, a decrease was noted with diaphragm tissue. Somewhat parallel to this is the decrease noted with diaphragm tissue after quinidine which produced no significant changes in heart respiration. It would therefore appear that these two types of muscles differ both qualitatively and quantitatively in response to these drugs at the dose levels employed. This may be explained as a difference in sensitivity and/or to drug distribution.

The relative insensitivity of rats to cardiac glycosides reported by others (32,38,41,77) is supported by our findings.

The increase in respiration of guinea pig brain slices in the HM flasks may indicate that the central effects of ouabain are mediated in part by an effect on the metabolism of brain tissue.

It is hazardous to endeavor to inter-relate the pharmacologic effects of these drugs on the heart with the effect on heart tissue respiration. Certainly in the light of present knowledge a cause and effect relationship cannot be established but it would seem possible that the effects of these drugs on the heart may be associated in some way with their influence on the metabolism of the heart tissue.

Considerable confusion exists in the literature concerning the action of these drugs on respiration (Table I page 13). This is due largely to variations in techniques. In most instances the drugs were added to a tissue from the side arm of a Warburg vessel often after the tissue had been respiring for a long time. The danger in applying results from such experiments to <u>in vivo</u> response are obvious. It is felt that our procedure of administering the drug to the intact animal

provides more pertinent data.

This work casts little further light on the role of ions and substrates in the response of tissue respiration to cardiac drugs which has been explored with contradictory results by others (32,38,41). However as a criterion for the results obtained in HM flasks two solutions were used, one rich in substrate (KMIII) and one a simple saline solution (KRP). Any inadequacy in the HM procedures should have been manifest.

It would appear from our results that the technique of determining tissue respiration in oxygen is a useful tool for pharmacologic studies. The level of response of normal animals was higher in this medium than in either of the liquid media and agrees within experimental limits with those reported by Huston and Martin (69). These authors reported a greater sensitivity for this method in examining the effects of dinitrophenol and sodium arsenite on tissue respiration. The greater sensitivity would also appear to obtain in our studies on cardiovascular drugs. Significant effects were shown by this technique which were not seen with the tissues in liquids (eg. ouabain effect on guinea pig heart and on guinea pig brain). Since the normal values were higher with the Huston-Martin technique it might be thought that whereas a depressant effect would be more readily manifest a stimulant one would not. This does not obtain since a more marked increase was seen in the case of guinea pig heart after ouabain, while a more pronounced depression was noted in guinea pig diaphragm after quinidine by intraperitoneal injection. These findings would indicate a greater general sensitivity. The inadequacies of the method are largely those inherent in any procedure where a tissue is taken from its milieu intérieur and examined in vitro.

IX. SUMMARY AND CONCLUSIONS

The drugs being investigated were injected into the animal, the tissues were removed and the oxygen consumption measured in Krebs Ringer Phosphate (KRP), in Krebs Medium III (KMIII) and in oxygen (HM flasks).

1. Ouabain by intraperitoneal injection:-

(i) At 45 mg./Kg. caused:-

(a) In rat heart a significant decrease in respiration in the HM flasks at zero time and sixty minutes thereafter.

(b) In rat diaphragm respiration a significant decrease in HM flasks at zero time and sixty minutes and a significant increase in rat diaphragm respiration at sixty minutes in KRP.

(ii) At 10 mg./Kg. caused:-

(a) In guinea pig heart slices a significant increase in respiration at zero time and sixty minutes in HM flasks.

(b) In guinea pig diaphragm oxygen consumption a decrease at zero time in all media.

(iii) At 0.5 mg./Kg. caused:-

(a) A significant increase in guinea pig brain in HM flasks at zero time.

(b) A significant increase in guinea pig heart at zero time in KMIII.

2. Digitoxin by subcutaneous injection:-

(i) At 30 mg./Kg. caused:-

(a) A significant depression in oxygen consumption of rat heart in HM flasks but only at sixty minutes.

(b) In rat diaphragm a significant increase in respiration at zero time in KRP, at sixty minutes in KRP and at sixty minutes in KMIII.

(ii)At 10 mg./Kg. produced:-

(a) No significant change in guinea pig heart or diaphragm respiration.

3. Quinidine Sulphate by intraperitoneal injection:-

(i) At 30 mg./Kg. produced:-

(a) A significant decrease in respiration of rat heart slices at zero time and sixty minutes in KRP and at sixty minutes in KMIII.

(b) A significant increase in rat diaphragm respiration in KRP at zero time.

(c) No change in respiration of rat diaphragm in KMIII or HM flasks at zero time but a depression in HM flasks at sixty minutes.

(ii) At 300 mg./Kg. produced:-

(a) A significant decrease in respiration of heart slices from rat at zero time and sixty minutes in KRP, at sixty minutes in KMIII and at sixty minutes in HM flasks.

(b) A significant decrease in respiration of rat diaphragm in all media at both times.

(c) A significant increase in KMIII at zero time and a significant decrease at sixty minutes in guinea pig heart slices in HM flasks.

(d) A depression of guinea pig diaphragm oxygen consumption in all media at both times.

4. Quinidine Sulphate by intramuscular injection:-

(i) At 300 mg./Kg. showed:-

(a) No significant change in guinea pig heart in any medium.

(b) A significant decline in oxygen consumption of guinea pig diaphragm at zero time in both KMIII and HM flasks and at sixty minutes in HM flasks.

5. The significance of the findings and the utility of the Huston-Martin technique are discussed.

X.BIBLIOGRAPHY

1. Goodman, L.S. and Gilman, A. The Pharmacological Basis of Therapeutics, 1955, 2nd ed., MacMillan.

2. Chen, K.K., Robbins, E.B. and Worth, H.J. J. Am. Pharm. A. (Scient. Ed.), 1938, 27, 189.

3. Jacobs, W.A. Physiol. Rev., 1933, 13, 222.

4. Stoll, A. The Cardiac Glycosides, The Pharmaceutical Press, London, 1937.

5. Grollman, A. Pharmacology and Therapeutics, 1954, 2nd ed., Lea and Febiger, 334.

6. Cattell, M. and Gold H. J. Pharmacol. Exper. Therap., 1938, 62, 116.

7. Heymans, C., Bouckaert, J.J. and Regniers, P. Comp. rend. Soc. de biol., 1932, 110, 573.

8. Gold, H., Kwit, N.T., Otto, H. and Fox, T. J. Clin.Invest., 1939, 18, 429.

9. Gold, H., and Modell, W. J. Pharmacol. Exper. Therap. 1932, 46, 357.

10. Starr, I., Gamble, C.J., Margolies, A., Donal, J.S. Jr., Joseph, N., and Eagle, E. J. Clin. Investigation, 1937, 16, 799.

11. Ferrer, M.I., Harvey, R.M., Werko, L., Dresdale, D.T., Cournand, A. and Richards, D.W. Jr. Am. Heart J., 1948, 36, 816.

12. Mendelstamm, M. Z. ges. Exptl. Med., 1926, 51, 633.

13. Belligheimer, E. Klin Wochschr., 1929, 8, 724.

14. Gold, H., Kwit, N. Science, 1937, 86, 330.

15. Schuntermann, C.E. Z. ges. Exptl. Med., 1935, 96, 520.

16. Salter, W.T., Sciarini, L.J. and Rubin, B. J. Pharmacol. Exper. Therap., 1949, 97, 314.

17. McGuigan, R.A. and Higgins, J.A. J. Lab. Clin. Med., 1938, 23, 839.

18. Lieberman, A.L. J. Pharmacol. Exper. Therap., 1933, 47, 183.

19. Nahum, L.H. and Hoff, H.E. Proc. Soc. Exper. Biol. Med., 1937, 36, 860.

20. Smith, P.K. , Winkler, A.W. and Hoff, H.E. Arch. Internal Med. 1939, 64, 322.

21. Nyiri, W. and DuBois, L. J. Pharmacol. Exper. Therap., 1930, 39, 111.

22. Ransom, F. J. Physiol. 1917, 51, 176.

23. Calhoun, J.A. and Harrison, T.R. J. Clin. Invest., 1931, 10, 139.

24. Wood, E.H. and Moe, G.K. Am. J. Physiol., 1941, 136, 515.

25. Wedd, A.M. J. Pharmacol. Exper. Therap., 1939, 65, 268.

26. Wood, E.H. and Moe, G.K. Am. J. Physiol., 1942, 137, 6.

27. Victor, J. Proc. Soc. Exper. Biol. Med., 1932, 30, 399.

28. Macht, D.I. Arch. int. pharmacodyn., 1951, 85, 321.

29. Gremels, H. Arch Exper. Path. u. Pharmakol, 1937, 186, 625.

30. Salomon, K. and Riesier, O. Arch. Exp. Path. u. Pharmakol., 1935, 177, 450.

31. Yavorsky, M.A. and Reif, E.C. J. Am. Pharm. Assoc., 1935, 24 No. 2, 108.

32. Wollenberger, A. J. Pharmacol. Exper. Therap., 1947, 91, 39.

33. Dunn, C.E., Holland, W.C. and Greig, M.C. Fed. Proc., 1954, 13, 350.

34. Lévy, J., Bull. Chim. Soc. Biol., 1946, 28, 328.

35. Lévy, J., Schwob, A. and Libert, O. Bull. Chim. Soc. Biol. 1946, 28, 647.

36. Finkelstein, M. and Bodanski, O. J. Pharmacol. Exper. Therap., 1948, 94, 274.

37. Lévy, J. and Schwob, A. Bull. Chim. Soc. Biol., 1946, 28, 642.

38. Herrmann, R.G. Arch. int. pharmacodyn., 1950, 81, 235.

39. Rothlin, E. and Schoelly, O. Helv. Physiol. et Pharmacol. Acta, 1950, 8, 69.

40. Nowy, H. and Helmreich, E. Klin. Wochsch., 1950, 28, 686.

41. Doull, J., Herrmann, R.G., Geiling, F.M.K. and Dubois, K.P. Arch. int. pharmacodyn., 1951, 86, 487.

42. Fischer, H., Huber, P., and Langemann H. Helv. Physiol. et Pharmacol. Acta, 1951, 9, 416.

43. Langemann, H., Brody, T.M. and Bain, J.A. J. Pharmacol. Exper. Therap., 1953, 108, 274.

44. Lee, K.S. J. Pharmacol. Exper. Therap., 1953, 109, 304.

45. Hisada, S. and Nakashima, J. Nagoya Med. J., 1953, 1, 277.

46. Herrmann, R.G., Flamboe, G.E. and Chen, K.K. J. Pharmacol. Exper. Therap., 1954, 112, 23.

47. Hunziker, H. Verteljahrsschr. naturforsch. Ges. Zurich 99 Beih. No. 2, 1954.

48. Burdette, W.J. J. Lab. Clin. Med., 1952, 40, 867.

49. Burdette, W.J. Am. Heart Journal, 1953, 46, 602.

50. Smith, J.A., Glassman, M., Lind, A.H., Post, M., Sohn, H., Warren, S. Proc. Soc. Exper. Biol. Med., 1954, 86, 747.

51. Liebig, H. Arch exper. Path. u. Pharmakol., 1940, 196, 137.

52. Bomskov, C., Nikolai, K., Kaulla, V. and Maurath, J. Arch Exper. Path. u Pharmakol., 1941, 198, 232.

53. Kimura, T.E., Proc. Soc. Expt. Biol. Med., 1948, 69, 419.

54. Segre, G. Arch. int. pharmocodyn. 1949, 80, 336.

55. Wollenberger, A. Arch. exper. Path. u. Pharmakol., 1953, 219, 408.

56. Casasús, A.F. Anales inst. farmacol. españ., 1954, 3, 213.

57. Reiter, M. and Barron, E.S.G. Arch. exper. Path. u. Pharmakol., 1952, 214, 341.

58. Kimura, T.E. and Dubois, K.P. Science, 1947, 106, 370.

59. Guerra, F., Eberstadt, P.L. and Veerkamp, A. Arch. inst. cardiologia. Méx., 1946, 16, 449.

60. Hegglin, R., Grauer, H. and Münchinger, R. Experimentia, 1949, 5, 127.

61. Helmreich, E. and Simon, K. Z. Naturforsch., 1952, 76, 341.

62. Langemann, H. Helv. Physiol. et Pharmacol. Acta., 1953, 11, C20.

63. Wollenberger, A. J. Pharmacol. Exper. Therap., 1951, 103, 123.

64. Grisolia, S. Biochim. Biophys. Acta, 1955, 18, 437.

65. Weese, H. and Wiegand, C. Medizin und Chemie (I.G. Farbenindustrie) Leverkusen 1934, 2, 148.

66. Wollenberger, A. Fed. Proc., 1950, 9, 326.

67. Webb, J.L., Saunders, P.R. and Nakamura, K. J. Pharmacol. Exper. Therap., 1951, 101, 287.

68. Uyeki, E.M., Geiling, E.M.K. and Dubois, K.P. Arch. int. pharmacodyn., 1954, 92, 191.

69. Huston, M.J. and Martin, A.W. Proc. Soc. Exper. Biol. Med., 1954, 86, 103.

70. Huston, M.J. and Martin A.W. Arch. int. pharmacodyn., 1955, 101, 349.

71. Umbreit, W.W., Burris, R.H. and Stauffer, J.F. Manometric Techniques and Tissue Metabolism 2nd Edition, Burgess.

72. Krebs, H.A. Biochim. Biophys. Acta, 1950, 4, 249.

73. Wollenberger, A. and Karsh, M.L. J. Am. Pharm. Assoc. (Sc. Ed.) 1951, 40, 637.

74. Johnson, L.P.V. An Introduction to Applied Biometrics, 1950, Burgess.

75. Kenney, J.F. and Keeping, E.S. Mathematics of Statistics Part One 3rd edition, D. Van Nostrand.

76. Griffith, J.Q. and Farris, E.J. The Rat in Laboratory Investigation, J.B. Lippincott.

77. Genuit, H. Arch. Expt. Path u. Pharmakol., 1938, 188, 285.

78. Sollmann, T. A Manual of Pharmacology, 1948, 7th Edition, W.B. Saunders.

79. Libert, O. Bull. Chim. Soc. Biol., 1946, 28, 771.

80. Rodnight, R. and McIlwain, H. Biochem. J., 1954, 57, 649.